Influence of soil water regime on nitrogen availability and plant competition in wet meadows

Thesis

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Thesis submitted for the degree of Doctor of Philosophy

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March 2005

Department of Biological Sciences
The Open University

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Page No.	Item/section redacted
3	Figure 1.1 A species-rich wet meadow in Cricklade, Wiltshire
3	Figure 1.2 (a, b and c) Some important wet meadow species

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Abstract

Identified ecological drivers controlling the equilibrium of species coexistence in wet meadows include site hydrology, soil nutrient availability and grazing. Of these, depth and annual variation of the water table has been considered as a primary factor and been frequently used in management decisions. A mechanistic understanding of how water regime influences species coexistence is thus vital for guiding conservation practices. In this context, this thesis explores the involvement of nitrogen availability, an often limiting resource which may be dependent on soil water regime. Laboratory and mesocosm experiments alongside field observations were undertaken to explore the interrelationships between water regime, nitrogen availability and plant competition. Three coexisting meadow species: meadow fescue, *Festuca pratensis*, common sedge, *Carex nigra* and greater burnet, *Sanguisorba officinalis*, were used to study the consequences in plant competition.

Study of soil nitrogen mineralization revealed a depression in mineralized nitrogen as matric potential approached zero. This depression coincided with soil air-filled pore space of less than 10%. The changes in water tension were accompanied by changes in soil microbial community composition as indicated by their phospholipid fatty acid signatures. Mesocosm study of *F. pratensis* and *C. nigra* grown on a gradient of constant water regime showed significant differences in biomass production and tissue nitrogen concentration. Individually and in competition the species responded by modifying resource allocation to reproductive/vegetative as well as shoot/root tissues. Nitrogen fertilization removed the influence of water regime on biomass production and tissue nitrogen concentration of *C. nigra* and *S. officinalis*. However, it did not significantly negate the influence of water regime on plant competitive response. Direct

field observation in a species-rich meadow confirmed species richness, biomass production and tissue nitrogen concentration were correlated to both soil water regime and nitrogen availability. A multivariate ordination of all recorded species along measured gradients of soil water regime, nitrogen availability and plant tissue nutrient concentrations indicated evidence of niche separation between species.

Parts of this thesis have been presented and abstracted:

Talks:

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CONTENTS

List of Tables

List of Figures

Chapter One

1 Introduction

This chapter introduces wet meadow conservation needs, the plant community used for the study and overall layout of the thesis.

1.1 Wet meadow conservation in the UK

Wet meadows are grassland ecosystems typified by seasonal shallow surface water and periodic anoxic soil conditions. Wet meadows form a transition between terrestrial and aquatic systems, where either the ground water-table is usually at or near the surface or the land is intermittently flooded from surrounding water bodies (Richardson, 2001). An example of wet meadow is shown in Figure 1.1.

Wet meadows are of high nature conservation value, supporting considerable biodiversity, including rare and threatened plant species and vegetation types, important bird populations and a range of invertebrates (See Figure 1.2). The botanical diversity in these ecosystems can reach up to 38 species per m^2 (Joyce and Wade, 1998). They also are known to provide refuge for globally threatened plants like *Apium repens* and *Selinum carvifolia* (Jefferson and Grice, 1998). Internationally important animals associated with wet grasslands in the UK include the corncrake bird *Crex crex* and the marsh fritillary butterfly *Eurodryas aurinia* (UK Steering Group, 1995).

In the UK, over the 50 years up to 1984, 97% of the area previously occupied by species-rich grasslands was lost (Fuller, 1987). These losses have continued, though at a slower rate, through the 1980s and 1990's (UK Steering Group, 1995). The main causes for this have been agricultural intensification, mainly application of artificial fertilizers, drainage and ploughing for arable production (Fuller, 1987; Minns *et al.*, 2001). Currently, the lowland floodplain hay meadow (*Alopecurus-Sanguisorba* community) has a total cover of less than 1000 ha (Jackson and McLeod, 2000). These occur in scattered sites from the Thames Valley through the Midlands and Welsh

borders to the Ouse catchment in Yorkshire. This particular wet grassland is protected by the EU Habitats and Species Directive (CEC, 1992).

Figure 1.1 A species-rich wet meadow in Cricklade, Wiltshire (Photo: Mike Dodd)

Figure 1.2 Some important wet meadow species: Snake's head fritillary (a), marsh fritillary (b), and corncrake (c) Photos: www.gartendatenbank.de and www.ukbap.org.uk

Since signing the Biodiversity Treaty at the Rio Earth Summit in 1994, the UK Government has set up Biodiversity Action Plans aimed at implementing measures for

conservation, restoration and expansion of important habitats. Both nationally and on a European scale, the lowland hay meadow has been recognised as a habitat of conservation importance and has been included in Annex 1 of the EC Habitats and Species directive (UK Biodiversity Group, 1998). In this connection, action plan objectives and proposed targets of the UK Biodiversity Action Plan include: arrest the depletion of species-rich habitats throughout the UK; initiate rehabilitation management for all significant stands currently in unfavourable condition by 2005; and by 2010 attempt to re-establish 500 ha of lowland hay meadow of wildlife value at carefully targeted sites.

1.2 Influence of hydrology and nitrogen availability on floodplain meadow communities

The plant community of semi-natural floodplain meadows is a unique assemblage, typically composed of a wide range of species. Identified ecological drivers controlling the equilibrium of species coexistence in this community include site hydrology, soil nutrient availability and grazing management (Berendse *et al.*, 1992; Gowing *et al*, 2002).

Of the above, the depth and annual variation of the water-table has been documented as the main factor influencing plant community composition by determining the relative competitive abilities of plant species (e.g. Hayati and Proctor, 1990; Gowing and Youngs, 1997; Castelli *et al.*, 2000). The influence of water-regime is such that management of target vegetation communities for nature conservation focuses on maintenance of appropriate water-regimes for certain periods of the year (Joyce and

Wade, 1998; Oomes *et al.*, 1996; Van Duren and Pegtel, 2000). Even minor shifts in soil moisture tension of less than 5 kPa (50 cm) have been shown to play significant ecological role on the composition of plant communities (Davies and Gowing, 1999; Silvertown *et al.*, 1999). Moreover, a switch in abundance of coexisting plant species distributions can occur over a range of as little as 1-2 kPa (10-20 cm). The sensitivity of these species to mild soil drying may not be necessarily explained by restricted water uptake, as these sites are well supplied with water. Hence other suggestions worthy of consideration are aeration, reduced diffusability of soluble nutrients, change in nitrogen mineralization or increased soil shear strength retarding root elongation (Pickett and Bazzaz, 1978; Davies and Gowing, 1999).

Field studies conducted as early as 1954, by the eminent German botanist Heinz Ellenberg, demonstrating that mild soil drying could control plant competition, had also brought to light the interaction of water-regime with nitrogen supply (Ellenberg, 1954 cited in Austin, 1990). Subsequent studies have shown the influence of water-regime on nitrogen mineralization (e.g. Stanford and Epstein, 1974; Myers *et al.* 1982). More recently strong suggestions on the involvement of soil related processes with water regime have been made (Neill, 1990; Levine *et al.* 1998). However, most studies so far have not specifically emphasised minor differences in moist soil or their influence on plant growth. This is particularly important as nutrient availability is known to determine the outcome of interspecific competition (Berendse, 1983; Austin, 1990) . In 1999, Aerts stated that there was a need for studies to link nitrogen availability and water-regime using integrated field and laboratory investigation.

The focus on nitrogen is not unprecedented, as nitrogen is a major plant nutrient, the supply and availability of which, often limits plant growth (e.g. Runge, 1983). Large

quantities of nitrogen are stored in organically-bound forms in floodplain meadows (Abbasi *et al.*, 2001). However the actual availability of nitrogen in soils depends on the rate of transformation of this organic nitrogen into plant available mineral forms (NH_4^+ and NO_3^-) and their transport through the soil (Antonopoulos, 1999; Jamieson *et al.*, 1999). These products of nitrogen mineralization are then rapidly absorbed by either plants or the microbial community. A schematic diagram of the nitrogen cycle and summary of the processes involved in a meadow context are given in Figure 1.3.

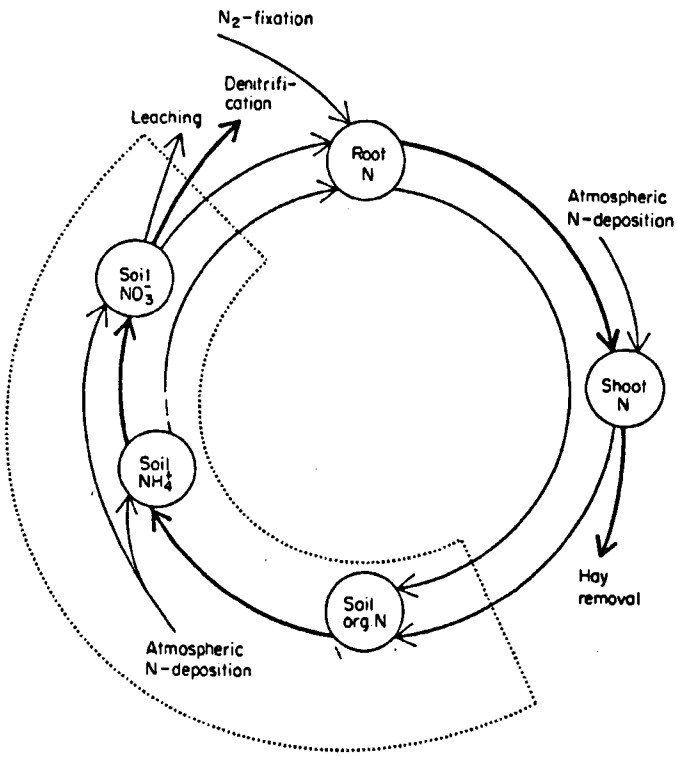

Figure 1.3 Simplified diagram of the nitrogen cycle in a meadow, with major inputs and outputs. The area bounded by the broken lines indicates soil processes. (after Berendse *et al.*, 1994)

The transformation of organic nitrogen into plant available mineral forms is referred to as nitrogen mineralization and is a microbial mediated process (e.g. Schimel and

Bennett, 2004). Nitrogen mineralization is influenced by a number of factors, the most important of which are soil moisture and temperature (e.g. Zak *et al.*, 1999). In a particular site, where large temperature variations due to water-regime are not expected or are accounted for, moisture remains the primary factor for investigation (Leiros *et al.*, 1999). Understanding the availability of nitrogen in response to water-regime, as well as its consequences for plant coexistence, would hence be a first step in guiding management for the conservation of wet meadows. While there is a substantial literature on cropping systems, few studies have addressed plant response to both soil drying and nitrogen availability in semi-natural grasslands (e.g. Berendse and Aerts, 1984; Grootjans *et al.*, 1985; Van Oorschot *et al.*, 2000).

This thesis will aim to elaborate on the effect of soil water-regime on soil-nitrogen availability and then follow-up on its consequences for plant response and how it affects competitive ability. An understanding of this mechanism is expected to provide useful information for the conservation of species-rich wet meadows (Olde Venterink *et al.*, 2001a; Critchley *et al.*, 2002).

1.3 Choice of plant community for the study

The focus of this study is the floodplain meadow community, labelled *Alopecurus pratensis-Sanguisorba officinalis* grassland or MG4 by the National Vegetation Classification (Rodwell *et al.*, 1992). The *Alopecurus-Sanguisorba* community is a seasonally-flooded lowland grassland on alluvial soils. It usually occurs on soils that are free draining, with neutral pH. This community has a species-rich and somewhat varied sward of grasses and herbaceous dicotyledons (Rodwell *et al.*, 1992).

For the mesocosm competition study, three typical MG4 species were used. The species were a sedge, *Carex nigra*, a grass, *Festuca pratensis* and a forb, *Sanguisorba officinalis*. The species were selected because they are known to coexist naturally in meadows yet have contrasting environmental requirements. This can be shown by their Ellenberg scores (Table 1.1). A brief description of the species is given below, along with pictures (Figure 1.4).

Table 1.1 Selected MG4 species and their Ellenberg scores[a]

Scientific Name	**F** *(Moisture)*	**R** *(pH)*	**N** *(Nitrogen)*
Common sedge *Carex nigra*	8	4	2
Greater burnet *Sanguisorba officinalis*	7	6	5
Meadow fescue *Festuca pratensis*	6	6	6

[a] The scores are given as calculated for the British Isles by Hill *et al.* (1999)

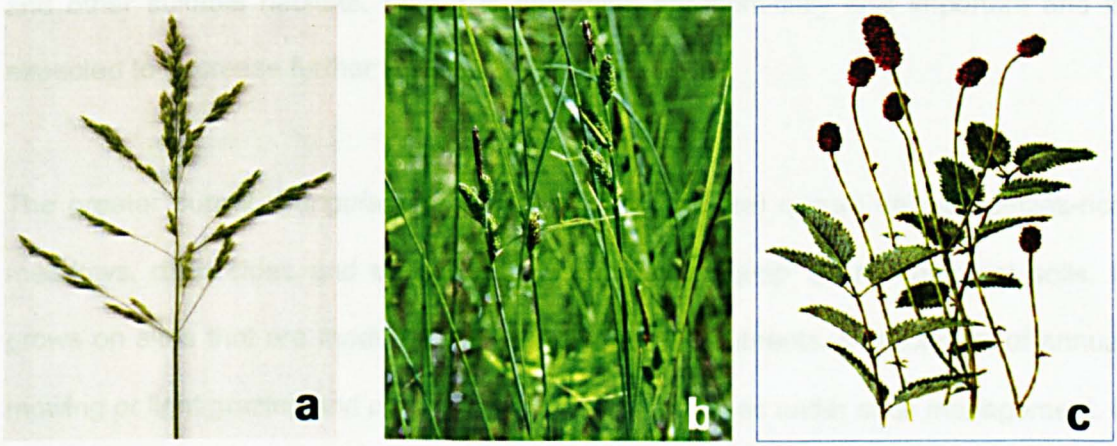

Figure 1.4 Diagram of the meadow fescue, *Festuca pratensis* (a) common sedge, *Carex nigra* (b), and greater burnet, *Sanguisorba officinalis* (c)

Photos: www.boga.ruhr-uni-bochum.de • www.uroweb.ru • www.floracyberia.net

The common sedge, *Carex nigra* (L.) Reichard (Cyperaceae) is an ecologically wide-ranging species. It is a rhizomatous, stress-tolerant sedge most typical of soligenous mire sites where the growth of potential dominants is suppressed by low fertility (and often also by grazing pressure). It also occurs beside upland streams, in moist grasslands and flood hollows, but is virtually absent from mire which is adjacent to open water. *Carex nigra* is still common in the UK, but decreasing as a result of habitat destruction (Grime *et al*, 1988).

The meadow fescue, *Festuca pratensis* Hudson (Poaceae) is a tufted, often relatively short-lived grassland species. The species is of agricultural importance, particularly in meadows. It is in many respects similar to *Lolium perenne*, but unlike *L. perenne*, is most frequent on moist to waterlogged soils and is less productive. Currently *F. pratensis* is decreasing as a native species due to the destruction of alluvial grasslands and other suitable habitats. Also it is becoming agronomically less important and is expected to decrease further (Grime *et al*, 1988).

The greater burnet, *Sanguisorba officinalis* L. (Rosaceae) occurs on old species-rich meadows, ditch sides and damp banks on moist to damp often winter-wet soils. It grows on sites that are moderately rich in bases and nutrients. It is tolerant of annual mowing or light grazing and competes well with tall grasses under such management. It is rather rare, and currently decreasing through habitat loss (Sinker *et al.*, 1991).

1.4 Aim and objectives of the study

This study will test whether the apparent correlation between species distribution and soil water-regime (Silvertown *et al.*, 1999) can be explained in terms of soil-nitrogen availability.

In this context the study has the following objectives:

1. Determine what changes occur in soil-nitrogen availability as a result of minor differences in soil water tension (0 – 10 kPa)

2. Determine the influence of water-regime and nitrogen availability on plant performance when growing in monoculture; and competition when growing in mixture

3. Describe the changes in meadow community composition in response to minor differences in water-regime

1.5 The study approach and organisation of the thesis

The investigations in this thesis were undertaken at three scales: controlled laboratory experiments, partially controlled mesocosm experiments and field monitoring.

The laboratory methods followed in this study, under controlled conditions, focused on the changes in soil physical and chemical characteristics as a result of changes in

water tension. The findings were then used to guide and supplement the experimental approach for both the field and mesocosm situations.

The mesocosm study aimed to recreate the field situation with limited controlled variables. It attempted to relate changes in soil-nitrogen due to minor differences in water-regime to the response of plants grown in monoculture and mixture.

In the field, plant distribution, productivity and soil nutrient availability were recorded along a gradient in soil water-regime. The field results were then compared with predictions from the fully and partially controlled laboratory and mesocosm experiments.

The main sections of this thesis by chapter are given as follows.

Chapter 2. Influence of fine-scale differences of water-regime on soil-nitrogen mineralization

In this section investigations into the effects of soil water potential on soil-nitrogen mineralization are described; including the involvement of soil aeration and the influence of soil temperature.

Chapter 3. Influence of soil water tension on plant growth, resource allocation and interspecific competition

In this chapter, the influence of imposed water-regimes on tissue nutrient concentration, resource allocation and competitive interactions between species is addressed.

Chapter 4. Influence of water-regime and nitrogen availability on the composition of a species-rich grassland community

In this section, soil-nitrogen availability and tissue nutrient concentration are monitored and relationships with water-regime analyzed. Furthermore, plant distribution and species-richness along a soil water-regime gradient is compared to soil-nitrogen availability.

Chapter 5. Integration: the involvement of soil-nitrogen availability on plant response

In this chapter, findings from the above three levels of investigation namely, chapters 2, 3 and 4 are synthesised. Included also are other relevant data and monitoring results.

Chapter 6: Conclusions

In this chapter the major findings are summarized and suggestions made for future study.

Chapter Two

2 Influence of water-regime on soil-nitrogen mineralization

This chapter deals on how subtle differences in water-regime influence soil-nitrogen mineralization. The effects of soil aeration and temperature are also investigated.

2.1 Introduction

Nitrogen is a major plant nutrient the supply and availability of which often limits plant growth and species competition in many natural environments (Tilman, 1982; Runge, 1983). Nitrogen mineralization, i.e. the microbially mediated transformation of organic nitrogen from soil organic matter into inorganic forms of ammonium and nitrate, is a key process for supplying nitrogen to plants (See Figure 2.1). Even though this mineralized inorganic nitrogen pool is usually very small relative to the total soil-nitrogen reserve, it forms the major source for plant uptake especially in environments that are not heavily impacted by fertilization or atmospheric nitrogen deposition (Abbasi *et al.*, 2001). For example, in unfertilised meadow ecosystems and heathlands in the Netherlands, nitrogen mineralization was shown to account for 70-80% of the nitrogen uptake by the vegetation (Berendse *et al.*, 1994). Knowledge of the potential ability of the soil to supply nitrogen for plant growth, and the factors influencing it has therefore been considered an important objective of ecological studies (e.g. Gonzalez-Prieto *et al.*, 1992; Schaffers, 2000).

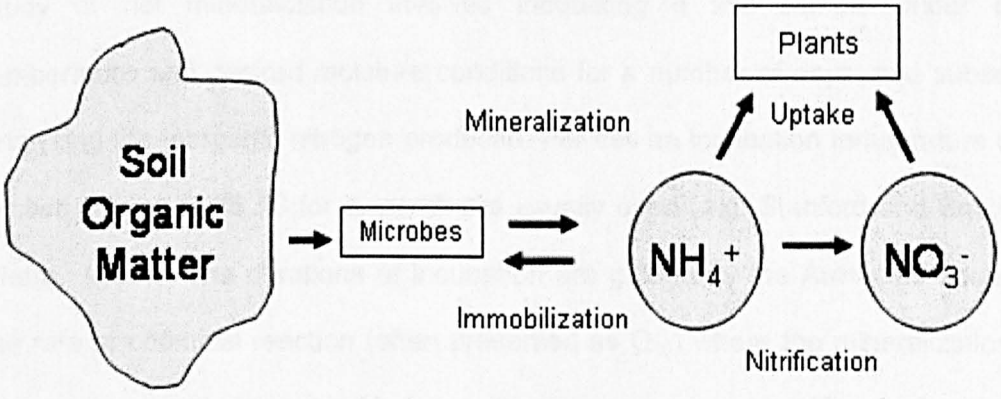

Figure 2.1 Conceptual scheme of soil-nitrogen cycle (after Schimel and Bennett, 2004)

Soil moisture is one of the most important and frequently studied environmental factors influencing mineralization (e.g. Pilbeam *et al.*, 1993; Paul *et al.*, 2003). Thus several authors have conducted studies on quantitative relationships between soil moisture content and nitrogen mineralization (e.g. Stanford and Epstein, 1974; Campbell and Paul, 1978; Myers, *et. al.*, 1982; Gonçalves and Carlyle, 1994; Paul *et. al.* 2003). However, few of these studies have experimentally investigated which aspect of soil wetness causes the changes in soil mineralization. This chapter reviews those results as well as experimentally investigating the driving factor behind the influence of soil moisture differences on soil-nitrogen mineralization.

2.2 Review of Earlier Studies

Net soil-nitrogen mineralization refers to the increase in soil inorganic nitrogen as a result of controlled incubation. It consists of the net balance between gross mineralization and microbial immobilization (Robertson *et al.*, 1999). Unless stated, instances of mineralization in this thesis refer to net mineralization. In the laboratory, study of net mineralization involves incubating a soil sample under constant temperature and desired moisture conditions for a number of days, and subsequently analysing the inorganic nitrogen produced. For this an incubation temperature of 35 °C for two weeks or 25 °C for a month are usually used (e.g. Stanford and Smith, 1974; Sierra, 1997). The durations of incubation are guided by the Arrhenius equation[1] for the rate of chemical reaction (often presented as Q_{10}) where the mineralization rate is assumed to double for every 10-degree increase in temperature (Stanford *et al.*, 1973).

[1] $k = A \times e^{-B/T}$ where k = rate, A & B are constants, and T is temperature (Sierra *et al.*, 1997)

In previous studies examining the relationships between soil moisture and nitrogen mineralization, soil moisture has been expressed in a number of different forms, such as gravimetric soil moisture content (e.g. Cameron and Kowalenko, 1976; Cassman and Munns, 1980); volumetric soil moisture content (e.g. MacDuff and White, 1985; Pilbeam *et al.*, 1993); soil water tension (e.g. Miller and Johnson, 1964; Sierra, 1997); or combinations of some of the above (e.g. Stanford and Epstein, 1974; Myers *et al*, 1982). Other indices used for soil moisture include percentage of field capacity[2] (Gonçalves and Carlyle, 1994; Leiros *et al.*, 1999) and water-filled pore space (%WFPS[3]) (Skopp *et al.*, 1990; Aulakh *et al.*, 1996; De Neve, 2002).

Many of the above studies of soil-nitrogen mineralization have been performed under an agricultural or forest setting and thus have focused on moist to dry soils i.e. moisture tension ranging between 30 kPa and few hundred kPa. Only few authors like Miller and Johnson (1964), Stanford and Epstein (1974) and Myers *et. al.* (1982) have described mineralization at soil moisture tensions < 10 kPa. Yet many natural ecosystems, for example wet meadows, experience variation in water tension of less than 10 kPa (Davies and Gowing, 1999). This highlights the need for understanding the effect of soil moisture on nitrogen mineralization at water tensions of less than 10 kPa and to investigate the factors driving it.

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[2] Field capacity refers to the water content of soil that has been allowed to drain freely for 48 hours from saturation with negligible loss due to evaporation (Townend *et al.*, 2001).

[3] % WFPS is used to refer to the proportion of the total soil porosity occupied by water. WFPS is calculated as: [gravimetric water content x (bulk density/total porosity)] x 100 (Linn and Doran, 1984).

A literature review of nitrogen mineralization collated from different agricultural soils, incubated under equivalent conditions, in response to varied soil water tensions is presented in Figure 2.2.

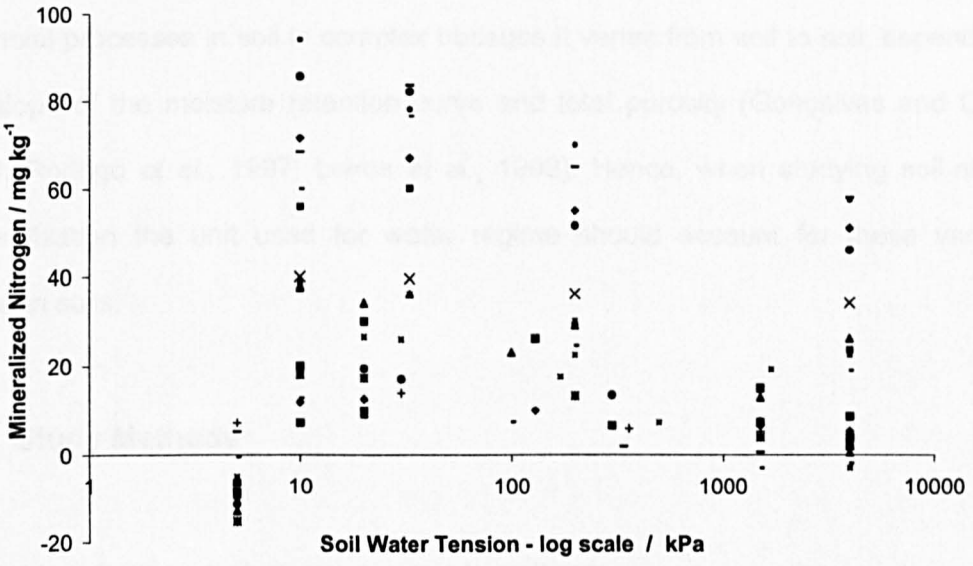

Figure 2.2 Mineralized nitrogen as a function of soil moisture tension on 20 soils (Stanford and Epstein, 1974; Myers *et al.,* 1982; Sierra, 1997). Similar symbols denote an individual soil studied under different tensions. Full data is given in Appendix 1.

Figure 2.2 shows nitrogen mineralization at its highest at ranges of about 10 – 30 kPa, and a decline on either side of this optimum, with the steepest decline occurring at the wetter end (lower tension). This range concurs to the 10 - 50 kPa reported by other investigators (e.g. Miller and Johnson, 1964; Reichman *et al.* 1966; Sabey, 1969; Cavalli and Rodriguez, 1975).

The response of mineralization is frequently linked to the activity of micro-organisms, which are responsible for converting organic nitrogen to inorganic nitrogen (Paul *et al.*, 2003). In dry soils mechanisms that cause a decrease in microbial activity include

reduced diffusion of soluble substrates, reduced microbial mobility and consequent access to the substrate (Zak *et al.*, 1999). In moist soils however, decreases in activity of aerobic microbes is usually attributed to oxygen deprivation caused by slow gas diffusion (Grant and Rochette, 1994). The relation between soil water content and microbial processes in soil is complex because it varies from soil to soil, depending on the slope of the moisture retention curve and total porosity (Gonçalves and Carlyle, 1994; Rodrigo *et al.*, 1997; Leiros *et al.*, 1999). Hence, when studying soil-nitrogen mineralization the unit used for water regime should account for these variations between soils.

2.3 Study Methods

2.3.1 Incubation study to measure mineralization

Laboratory incubation methods are useful for measuring the potential ability of the soil to supply nitrogen for plant growth and also for studying the factors influencing nitrogen availability (Robertson *et al.*,1999). Hence they have been used in a number of ecological studies, including grasslands (e.g. Grootjans *et al.*, 1985; Gonzalez-Prieto *et al.*, 1992; Schaffers, 2000; Van Oorschot *et al.*, 2000).

Earlier studies on the influence of soil moisture on nitrogen mineralization relied on creation of the desired soil moisture level by addition of known weight of water, based on inferences made from the soil moisture retention curve. This approach is not effective for attaining accurate minor water tension differences. This is because for soils close to saturation, the differences in water tension are very small and hence

interpolating weight differences is not reliable (De Neve and Hofman, 2002). Because of this reason gravimetric methods were not used.

For this study, soil water potential was used to describe soil moisture status as it is more discriminatory between wet soils and it also enables comparison of water availability in soils with different textures (De Neve and Hofman, 2002).

Two soils were used for studying nitrogen mineralization in response to differences in soil water tension. The first soil was an experimental loam made by mixing agricultural soil from Silsoe (Frilford soil series, Bedfordshire, UK), with peat moss (B&Q® Garden Peat, Chandlers Ford, UK) and sand (Silvaperl® Sharp sand, William Sinclair Horticulture Ltd., Lincoln, UK), in a ratio of 1:1:2. The second soil was sampled from a species-rich wet meadow in East Cottingworth (Fladbury soil series, Yorkshire, UK) using brass cores of 5 cm diameter and depth (98 cm^3) volume. The physical and chemical characteristics of the two soils are given in Tables 2.1 and 2.2.

For the experimental soil, the loam was used to fill a core at a bulk density of 1.2 g cm^{-3} and studied with 3 replicates for each water tension level. The meadow samples were kept in the cores they were sampled with to avoid disturbing their structure. The samples had an average bulk density of 0.65 g cm^{-3} and were incubated in 5 replicates for each water tension level.

Table 2.1 Some physical and chemical characteristics of the soils used

Soil Type	Soil Textural Class	pH	C (%)	N (%)	C:N	Extractable Potassium (mg kg^{-1})	Extractable Phosphorus (mg kg^{-1})
Experimental	*Loamy sand*	8	2.1	0.11	19.1	65	65
Meadow	*Clay loam*	5.8	8.9	0.60	14.8	98	9

Table 2.2 Soil water contents and air-filled porosity at different water tensions

Soil Type	Soil Water Tension (kPa)	0	0.5	1	2	3	4	5	6	7	8	9	10
Experimental	Soil water content (% volume)	44	39	37	29	28	26	20	20	18	18	16	19
	AFP [a]	0	5	7	15	16	18	24	24	26	26	28	25
Meadow	Soil water content (% volume)	76	71	66	60	39	44	41	-	47	-	47	46
	AFP	0	5	10	16	37	32	35	-	29	-	29	30

[a] AFP refers to air-filled porosity – (for calculation see section 2.3.4)

Water tensions were achieved by subjecting saturated soil cores to set tensions between 0 – 10 kPa on a sand suction table (Figure 2.3). The cores were left on the sand suction table for 48 hours until constant mass (Townend *et al.*, 2001), after which the cores were removed and sealed in an air-permeable laboratory parafilm (Parafilm M® Pechiney Plastic Packaging, Chicago, IL), and incubated at 35 °C for two weeks.

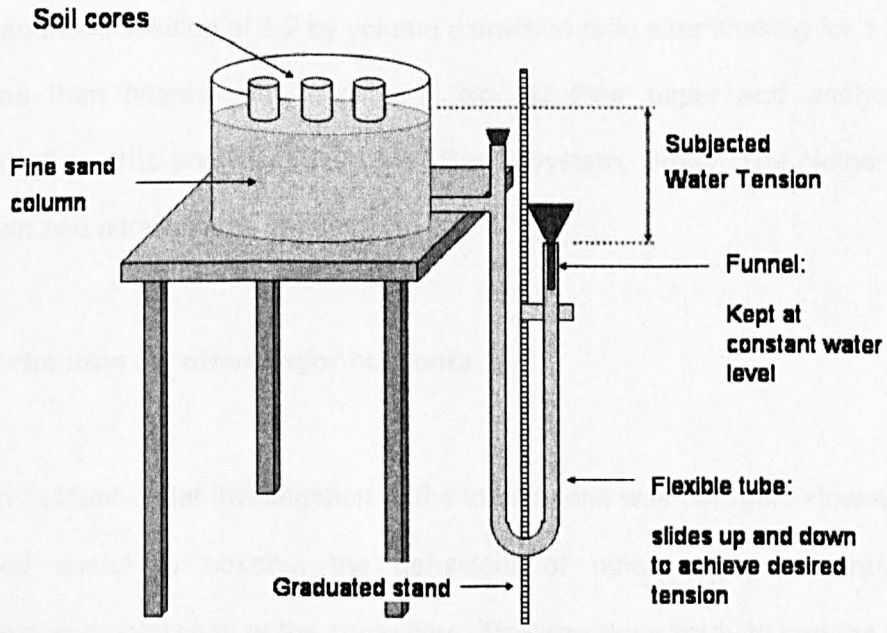

Figure 2.3 Diagram of the sand suction table used for subjecting soils to desired tension

To determine the influence of incubation temperature on nitrogen mineralization, a parallel experiment was run with temperatures of 4, 6, 8, 12, 18, 25 and 35 °C at 2.5 kPa tension. To clarify the influence of aeration on nitrogen mineralization, an additional experiment was run with water tensions between 0.5 – 2.5 kPa water tension, where soil pore space was manipulated. This was done by either compacting the soil to reduce pore space or mixing polypropylene pellets to increase total pore space (Robertson *et al.*, 1999).

Following incubations, the soils were removed from the cores and stored at -20°C before extraction. Freezing samples is a standard technique and routinely used to inhibit mineralization prior to extraction. As long as samples are thawed rapidly, i.e. less than 4 hours before extraction, there is no significant change in extracted nitrogen (Esala, 1995). The soils in this study were thawed for an hour and then extracted using 2 M KCl aqueous solution at 1:2 by volume extraction ratio after shaking for 1 hour. The slurry was then filtered with Whatman® No. 42 filter paper and analysed using continuous flow auto-analyser (SKALAR® San^{++} System, Breda, The Netherlands) for ammonium and nitrate forms of nitrogen.

2.3.2 Extraction for other major nutrients

The main nutrient under investigation in the incubations was nitrogen. However, it was considered useful to observe the behaviour of other major nutrients, namely phosphorus and potassium at the same time. This was done firstly to see the behaviour of the other nutrients and secondly to make sure they were available in adequate quantity.

Extractable soil phosphorus was determined using Olsen's reagent (MAFF, 1986). Five grams of air-dried soil was mixed with 100 ml of sodium bicarbonate reagent, shaken for 30 minutes and then filtered through Whatman® No. 42 filter paper. For colour complex analysis, 5ml of the sample extract was mixed with 1 ml of sulphuric acid and 25 ml of ascorbic-ammonium molybdate solution. This was allowed to stand for 30 minutes for the colour to develop, and then analysed through Helios® thermo spectroscopic colorimeter at 880 nm wavelength.

Extractable soil potassium was determined according to methods of MAFF (1986). Ten grams of air-dry soil passed through 2 mm sieve was shaken with 50 ml of NH_4-acetate for 30 minutes. The slurry was then filtered using Whatman® No. 42 filter paper and the extract analysed using Gallenkamp® SGA_330C (Gallenkamp, Loughbourgh, UK) flame photometer.

2.3.3 Incubation study for soluble organic nitrogen

Recently the role of soil amino acids as a potential source of nitrogen for certain plants, including that of grasslands has been considered (e.g. Streeter *et al.*, 2000; Endres and Mercier, 2003; Schimel and Bennett, 2004).

In light of this, it was decided to measure the concentration of amino acids in the laboratory incubated experimental soil samples. Soil cores were prepared in a similar fashion as for the incubation mineralization study and incubated at 18°C for 6 weeks.

Total Free amino acids (TFAA) were determined according to the methods of Streeter *et al.* (2000). A sample of 10 g of fresh soil was extracted with 75 ml of double-

deionized water (at 18 mohms resistivity) for 10 minutes in acid-washed polyethylene bottles under slow shaking action. Extracts were passed through a glass-fibre filter and filtrates were frozen at -20°C until further analysis. During analysis, amino acid concentrations were measured using high performance liquid chromatography (HPLC) using WatersNovaPak C18® cartridge (Waters® High Performance Liquid Chromatography, Milford, Massachusetts, USA).

2.3.4 Study of soil aeration

Soil aeration is an important factor in the study of soil nutrient cycles, as it controls microbial processes and chemical transformations (Paul *et al.*, 2003). The aeration status of the soils in this study was described through the concept of air-filled pore-space and redox potentials.

Air-filled porosity (AFP) is the fraction of the total soil volume that is occupied by air. Air-filled porosity is often used as an indicator of the aeration status of the soil and its ability to conduct and store gases (Ball and Smith, 2001). Total porosity (TPS) is the percentage of soil volume not occupied by solids. In moist soils, AFPS is obtained as the difference between TPS and the volumetric water content (V).

$$AFPS = TPS - V$$

The above calculation assumes no shrinkage or swelling occurs on the soil under study.

Redox potential in soils is determined as the potential created by oxidation-reduction reactions that take place on the surface of a platinum electrode measured against a reference electrode (Patrick *et al.*, 1996). Redox potential in this experiment was measured by placing a redox probe in intact cores after incubating for a week.

2.4 Results

2.4.1 Inorganic nitrogen

The response of soil-nitrogen mineralization for the experimental loam to differences in soil water tensions is given in Figure 2.4. Figure 2.5 shows the same for the meadow soil.

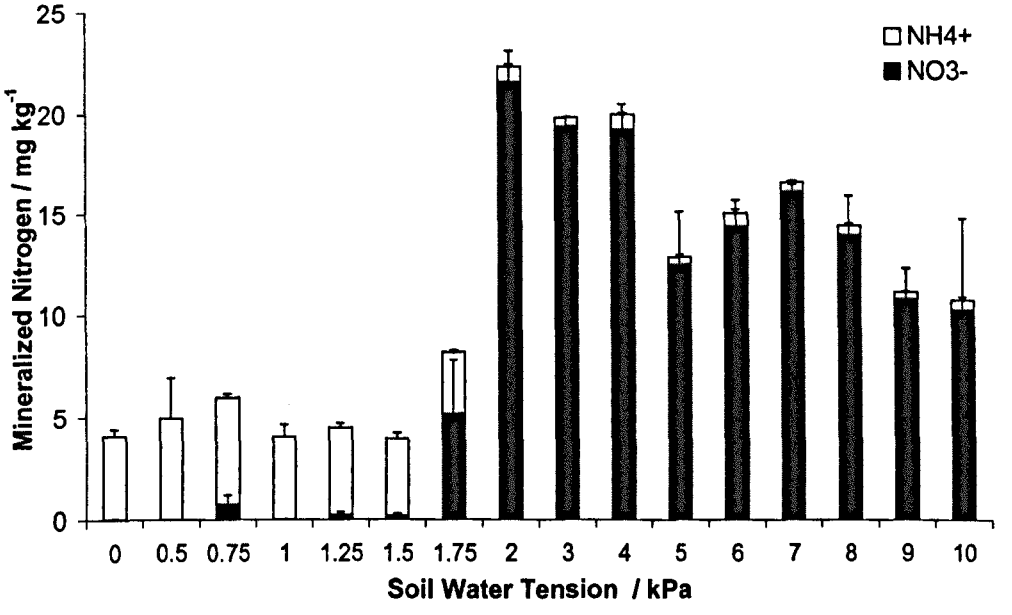

Figure 2.4 Nitrogen mineralization of the experimental loam under varied soil water tensions. Soils were incubated at 35 °C for 2 weeks. Bars indicate standard error.

The result for the experimental soil as given in Figure 2.4 shows a distinct depression of nitrogen mineralization at tensions below 2.0 kPa. At soil water tensions of more than 2.0 kPa, mineralized nitrogen declined, at a slower rate of 1.3 mg kg^{-1} for each 1.0 kPa drop in tension ($r = 0.88$, $p < 0.001$).

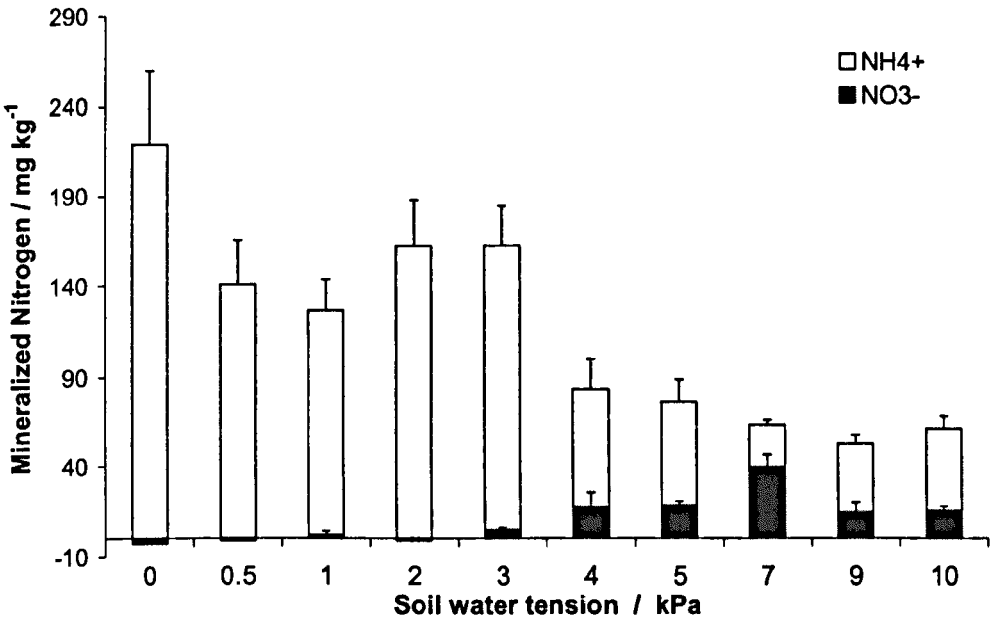

Figure 2.5 Nitrogen mineralization in meadow soil under varied soil moisture tensions. Soils were incubated at 35°C for 2 weeks. Bars indicate standard error.

The response of nitrogen mineralization from the meadow soil shows a decline in mineralization at higher tensions, at a rate of 15.66 mg kg^{-1} for each 1.0 kPa drop in tension ($r = 0.85$ $p < 0.001$). It may be noted that overall there is much higher amount of mineralized nitrogen and ammonium is the dominant form. Moreover, at the lower soil water tensions no depression in mineralization is observed.

2.4.2 Soil organic nitrogen

The chromatographs obtained from the High Performance Liquid Chromatography did not show any significant presence of free amino acids from any of the incubated experimental soils (See Appendix 2).

2.4.3 Soil aeration

The response of soil-nitrogen mineralization against air-filled porosity is shown in Figure 2.6. The air-filled porosity readings were calculated from the cores used for the aeration experiment.

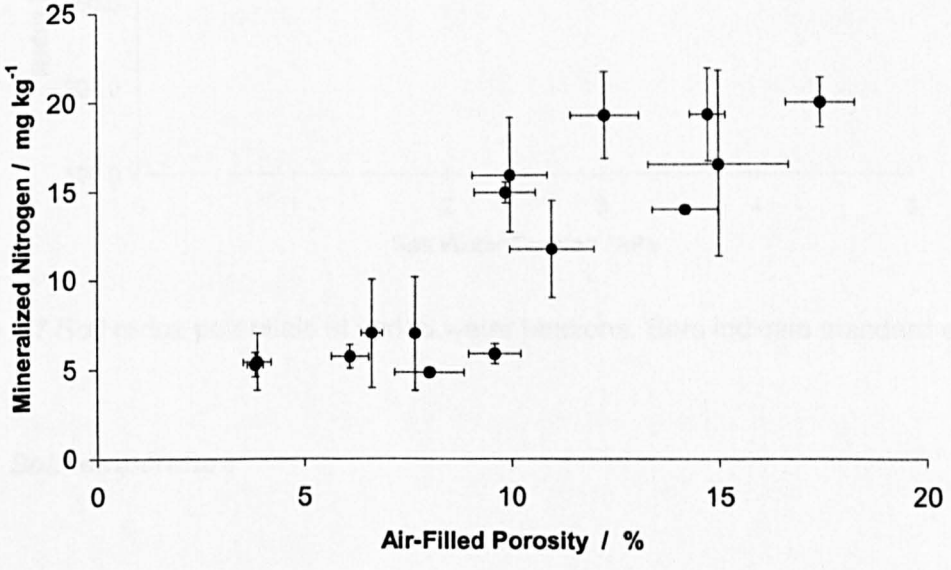

Figure 2.6 Air-filled porosity versus mineralized nitrogen under varying soil moisture tensions. Soils were incubated at 35°C for 2 weeks. Bars indicate standard error.

The regression of air-filled porosity against nitrogen mineralization showed a significant relationship (r^2 = 0.74, $p < 0.001$). A step change favouring nitrogen mineralization is observed when air-filled porosity exceeds 10%.

Soil redox potential readings were found to increase linearly from 282 mV at 0 cm water tension to 358 mV at 2.5 kPa soil water tension (See Figure 2.7). Beyond 2.5 kPa the redox potential readings appear to plateau.

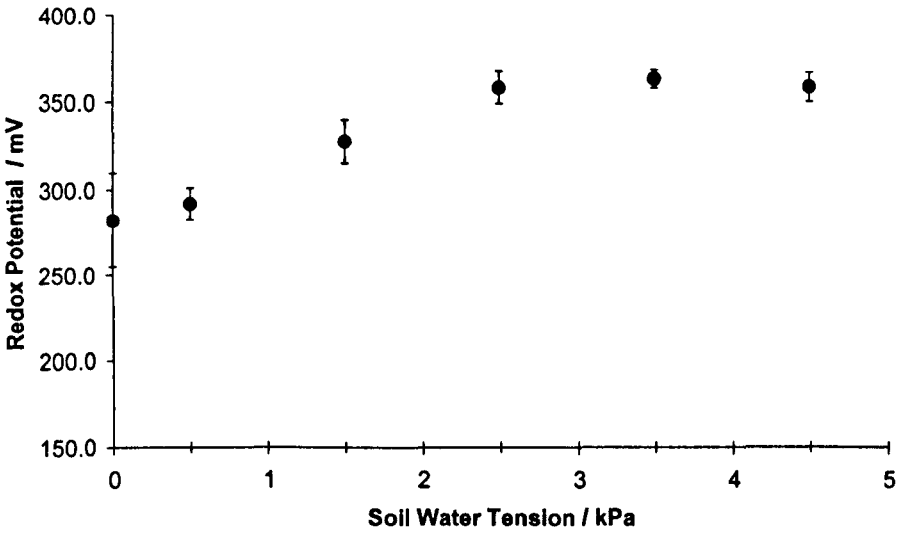

Figure 2.7 Soil redox potentials at varied water tensions. Bars indicate standard error.

2.4.4 Soil temperature

The rate of soil-nitrogen mineralization per day at different incubation temperatures ranging between 4 - 35 °C is shown in Figure 2.8.

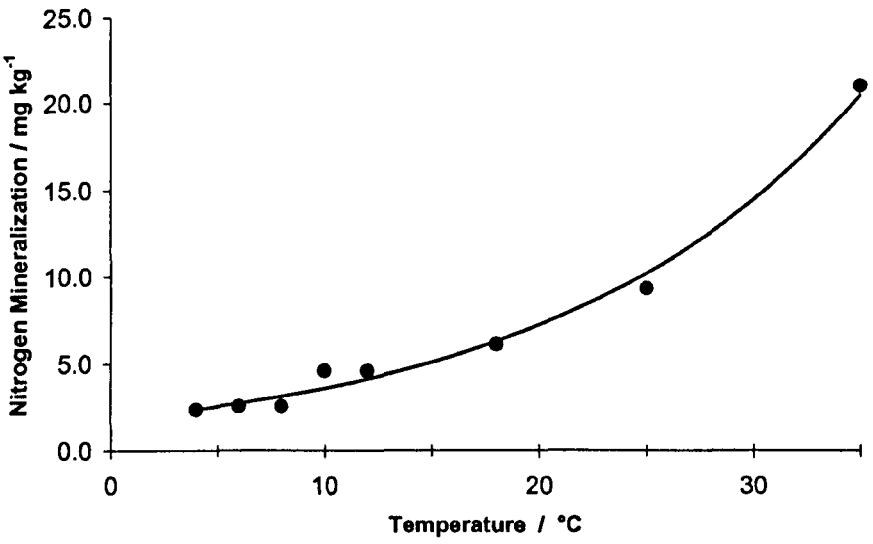

Figure 2.8 Nitrogen mineralization as influenced by incubation temperature

An exponential regression on the rate of soil-nitrogen mineralization showed Mineralization = $1.784*e^{0.0697*Temperature}$. This showed doubling of mineralization for every 10 °C increase in the incubation temperature (r^2 =0.97, $p < 0.001$).

2.4.5 Other major nutrients

The response of extractable phosphorus and potassium to differences in soil water tension is shown in Figure 2.9. The response shows that the two nutrients are not affected by incubation at varied soil water tensions.

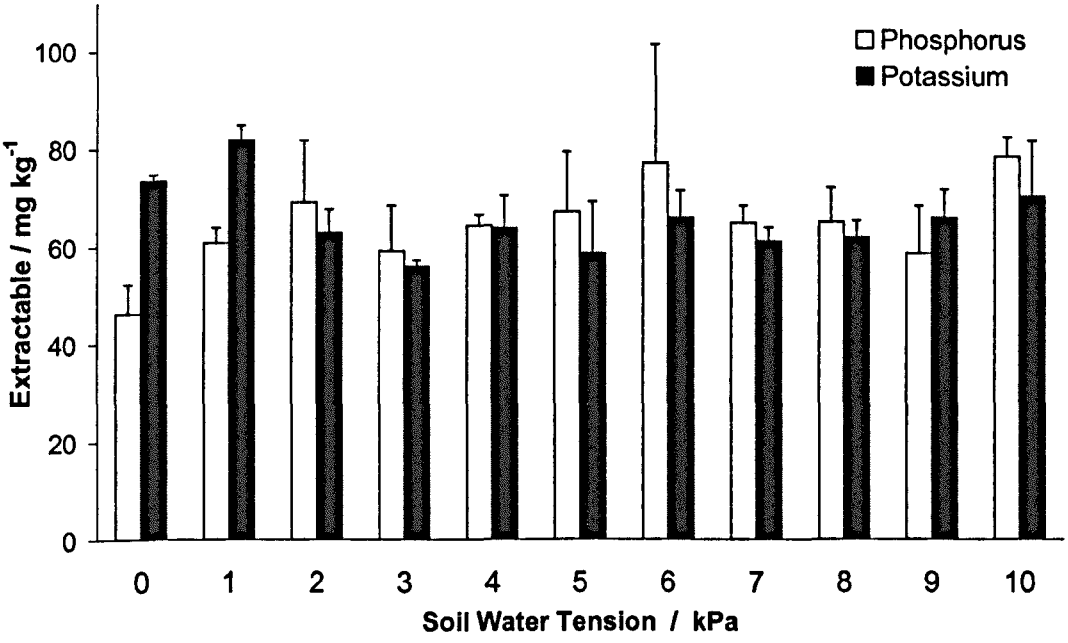

Figure 2.9 Extractable soil phosphorus and potassium in response to soil water tension. Bars indicate standard error.

2.5 Discussion

Mineralization is a largely microbial mediated process. The micro-organisms responsible for converting the organic nitrogen to inorganic forms are highly influenced by soil moisture and temperature (Zak *et al.*, 1999). The result of the experimental soil incubation (Figure 2.4) showed a distinct depression of nitrogen mineralization at tensions below 2.0 kPa and a slow linear decline above 2.0 kPa. The decline in nitrogen mineralization at the drier end i.e. > 2.0 kPa could be explained by the overall decrease in soil moisture availability, which results in decrease in microbial growth (Grant and Rochette, 1994). However below the 2.0 kPa tension, soil moisture is not a limiting factor and hence the need to focus at other possible factors.

On the other hand, when one examines mineralization of the meadow soil at low soil water tensions (Figure 2.5), the pattern does not seem to follow that of the mineralization from the experimental loam soil. This was unexpected and is considered to have arisen due to the existence of many plant roots in the field soil cores used for the incubation. The decomposition of roots, especially under anaerobic conditions is known to produce ammonium (Dorland *et al.*, 2003). A similar pattern but with high nitrogen mineralization at zero tension (saturation) had also been observed by Miller and Johnson (1964) who suggested this observation a result of particular type of micro-organisms functioning at this tension. However, beyond this wet end, as the soil water tension increased nitrogen mineralization of the meadow soil declined in a similar fashion to that of the experimental loam.

To understand the depression of nitrogen mineralization at low tension where soil moisture is not limiting, the examination of soil aeration is one possibility. Since aeration is recognised to be an important factor influencing mineralization (Miller and Johnson, 1964). Within this narrow range of soil moisture availability, it was possible to see a pattern of increase in nitrogen mineralization as air-filled porosity increases, as well as depression of mineralization when air-filled pore space is less than 10% (Figure 2.6). This is most likely due to reduced diffusion rate of oxygen, resulting in oxygen stress (Whalley *et al.*, 2000). This observation is also supported by the redox potential measurements, which increase until water tensions of 25 cm and plateau afterwards (Figure 2.7). Values of < 300 mV are generally accepted to signal anaerobic conditions (Gambrell *et al.*, 1991 in Dwire *et al.*, 2004). This is an indication that there is no aeration limit beyond tension of 2 kPa.

The aeration observation is also supported by earlier studies. Miller and Johnson (1964) observed that maximum mineralization was observed when air occupied > 20% of the total soil pore space, while Stanford and Epstein (1974) further noted this to be 10 - 20% (i.e. when water occupied at least 80-90% of the pore space).

The availability of the other major nutrients investigated, namely phosphorus and potassium did not respond to the differences in soil water tension (Figure 2.9). This may be explained by the knowledge that unlike nitrogen, phosphorus and potassium depend more on chemical processes for their availability and not on microbial mineralization (Helmke and Sparks, 1996; Lajtha *et al.*, 1999).

In this study, only trace total free amino acids were encountered, not any different from the blank standards used. This absence or low level is known especially in agricultural soils when there are high levels of inorganic nitrogen available (Owen and Jones, 2001). The absence of significant quantities of amino acids may also be due to them being immobilized by micro-organisms. Jones *et al.* (2004) provide evidence of this by documenting an increase in microbial respiration following immobilization. Experimental studies by Bardgett *et al.*, (2003) have shown that this immobilization to be very rapid, for e.g amino acids added to intact cores of unproductive semi-natural grasslands were almost totally sequestered within 50 hours. However, in the long run, this immobilized nitrogen may be subsequently mineralized and provide a useful source of nitrogen (Weigelt *et al.*, 2003).

The response of soil-nitrogen mineralization to incubation temperature showed an exponential rise (Figure 2.8). The rate followed that of the Arrhenius equation (Q_{10}) which has also been shown in many soil mineralization studies, most recently by

Heumann and Böttcher (2004) and Wang *et al.* (2004). The importance of differences in field soil temperatures hence can not be over emphasised. However in field situations it is unlikely there will be large differences in temperature as a result of the differences in water tension used in this study. Further evidence in support of the above statement from a 9 month mesocosm monitoring of soil temperature at ambient field conditions is discussed in Chapter 5, section 5.2.2.

2.6 Conclusion

Overall this investigation revealed a marked depression in mineralized nitrogen at wet soil conditions, except in the meadow soils with roots. This decline in mineralization coincided with an air-filled porosity of < 10 %. A slower decline in drier soils was also observed beyond an optimum range, which could be associated with limited soil moisture. The involvement of aeration in determining nitrogen mineralization strongly suggests that it is associated with microbial functioning. So far although microbial mediation has been frequently referred to and acknowledged, few studies have experimentally shown its involvement in relation to mineralization (e.g. Miller and Johnson, 1964). Further evidence elaborating this suggestion is detailed in Chapter 5 section 5.2.1. On the other hand, other major nutrients, phosphorus and potassium did not show any response to minor differences in water regime.

The response of nitrogen mineralization to differences in water-regime will potentially then have an impact on plant uptake and community composition (Vermeer and Berendse, 1983; Olde Venterink *et al.*, 2001a). In this context, flood meadows, where the soils are subjected to wet situations and where availability of nitrogen is a major limiting factor are such an example to be studied.

Chapter Three

3 Influence of water-regime on plant growth, resource allocation and interspecific competition

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In this chapter plant responses to differences in water-regime are described. Plant aboveground growth as well as root to shoot resource allocation are discussed. Plant competitive interactions at different water-table depths and the involvement of nitrogen are also mentioned.

3.1 Introduction

The importance of interactions between plants in determining the structure and dynamics of plant communities is widely recognized (e.g. Tilman, 1988; Grace and Tilman, 1990; Grime, 2001).

In such interactions the competition is one concept used to describe the relationship between plants. According to Grime (2001), competition is defined as the tendency of neighbouring plants to utilise the same quantum of light, ion of mineral nutrient, molecule of water or volume of space. In any plant interaction study it is hence necessary to identify for which resource the plants are competing (Aerts, 1999).

A framework plant interaction model proposed by Goldberg (1990), shown in Figure 3.1 formed the theoretical basis for the competition study in this thesis.

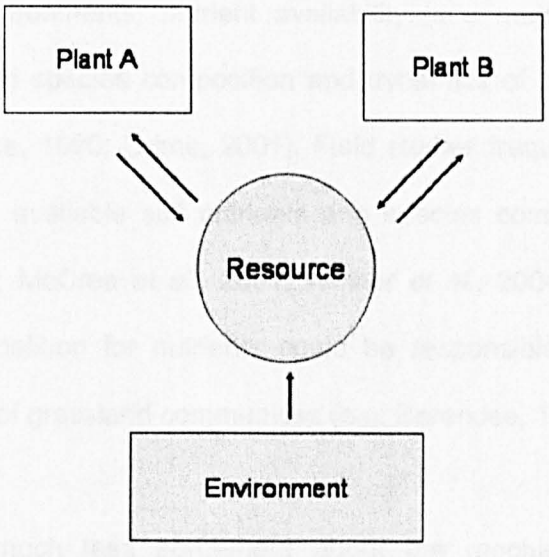

Figure 3.1 Plant competition model (after Goldberg, 1990)

This framework is based on the observation that most interactions between individual plants actually occur through some intermediary such as resources, pollinators, dispersers, herbivores or microbial symbionts. Such indirect interactions consist of two distinct processes: one or both plants has an *effect* on abundance of the intermediary and a *response* to changes in abundance of the intermediary.

At this juncture, a clear distinction between resources considered as an intermediary versus other potential environmental gradients is required. Environmental gradients could be indirect, where the environmental variable does not have a direct physiological influence on plant growth (e.g. altitude) or direct where resources have direct physiological influence in plants but are not consumed (e.g. pH, temperature). The intermediaries are resources that have direct physiological influence on the plant growth and are essential resources consumed by plants (e.g. nutrients) (Austin, 1990).

In many natural environments, nutrient availability is a major factor limiting plant performance, affecting species composition and dynamics of plant communities (e.g. Berendse and Elberse, 1990; Grime, 2001). Field studies frequently reveal significant correlations between available soil nutrients and species composition (e.g. Vermeer and Berendse, 1983; McCrea *et al.*, 2001; Weiher *et al.*, 2004). Hence, it has been suggested that competition for nutrients could be responsible for the regulation of species composition of grassland communities (e.g. Berendse, 1983; Austin, 1990).

There is however much less agreement about the mechanisms of interspecific competition (Aerts, 1999). With a view to resolving such questions, Tilman (1990) suggests that simple but powerful mechanistic models, which summarise parameters and processes, could be developed to study competition. This could then provide a

theory involving the intermediate resource, to predict how one individual affects another during competition and the outcome of interaction. This may further be elaborated for each plant species as competitive ability, which is defined for the purposes of the C-S-R[4] theory as a function of the area, the activity, and the distribution in space and time of the plant surfaces through which resources are intercepted. This as such depends upon a combination of plant characteristics for resource allocation and reproduction such as storage organs, lateral spread, growth rate and leaf nutrients (Grime, 2001).

Water in wet meadow systems is not considered a limiting resource; but rather as a direct environmental gradient, as it is available for most of the season in sufficient quantities. Nevertheless, plants growing in such environments show a clear distribution along a gradient in water-regime (Gowing and Spoor, 1998). One suggestion for this had been that the effect of water-regime is manifested via nutrients (Davies and Gowing, 1999). In this respect, nitrogen availability is considered as key resource, not only because nitrogen is the major plant nutrient, but also as it has a recognised dependence on soil moisture (as shown in Chapter 2). The role of nitrogen is indirectly strengthened from the evidence of studies conducted on sand culture (Figiel *et al.*, 1995; Güsewell *et al.*, 2003; Lodge *et al.*, in prep.). Use of pure sand growth media where nutrients are supplied externally means water-regime no longer exercises control on nitrogen mineralization. Hence it is not surprising that water-regime did not interact with nutrient supply in determining plant response. This led Güsewell *et al.* (2003) to suggest the possible involvement of other factors in nutrient availability through interaction of flooding with microbial community activities e.g. symbiotic nitrogen fixation (Bordeleau and Prévost, 1994) or mycorrhizal infection (Miller *et al.*, 1999).

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[4] Competitive-Stress tolerator-Ruderal theory of Grime (2001)

This study therefore looked at how plant response and competition is influenced by small differences in water-regime and the involvement of nitrogen availability through a partially controlled mesocosm study.

3.2 Materials and Methods

Simplified artificial communities and greenhouse experiments are suitable for competition studies due to the high degree of experimental control possible, repeatability and amenability to rigorous statistical design (Gibson *et al.*, 1999). Such studies also allow the study of the mechanisms of interaction *i.e.* through root and shoot capture of resources as well as the separation of different components of species interaction, such as *effect* and *response* (*sensu* Goldberg 1990) and determination of relative efficiency (Connolly *et al.*, 1990). Nevertheless some limitations do exist, one in particular being the restricted ability to apply the results of such experiments to complex natural communities. Despite this, unless plant interactions can be demonstrated under controlled greenhouse conditions, they are unlikely to be of importance to natural communities (Gibson *et al.*, 1999).

3.2.1 Controlled water tension system: Principles

Soil water status is an important environmental factor affecting several soil and plant processes. Hence maintenance of a constant soil moisture tension over an extended period of time, with actively growing plants, under controlled experimental conditions is a requirement.

A number of systems to maintain constant water tension have been developed for growth cabinet and greenhouse applications, most in the past 20 years. Some growth cabinet methods used have been: irrigation using porous steel tubes (Cao and Tibbitts, 1996; Steinberg & Henninger, 1997); or continuous circulating water under negative pressure (Lipiec *et al.* 1988; Iwama *et al*; 1991). Snow and Tingey (1985) and Wookey *et al.* (1991) worked on simpler versions whereby plant pots are suspended over a water column of known depth. A capillary mat irrigation system was used under greenhouse condition by Hoffman *et al.*, (1996). Also under greenhouse conditions, Mueller-Dumbois and Sims (1966) used a container resting inclined over a source of water, thus creating numerous water-table depths over the whole length of the plane. At a mesocosm scale, turf was grown on a fine sand column with drainage holes fitted at the required depths (Berendse & Aerts, 1984) and water supplied via a piezometer on a daily basis (Van Oorschot *et al.*, 2000).

Often the circulating water and irrigation growth cabinet systems are expensive to construct and maintain. In addition, some irrigation methods require uniform aggregate ceramic substrate instead of soil for proper operation, while the circulating water systems have difficulties in maintaining water tension for extended periods of time without siphons breaking down. Finally, the growth cabinet and greenhouse methods in the above examples do not lend easy applicability for use outdoors *i.e* under mesocosm situations with constant exposure to the weather. On the other hand the mesocosm systems reviewed require daily external water supply and hence do not guarantee constant water-table depth at all times.

The system in this study was developed to overcome some of the above problems by maintaining constant water-tables over a long duration of time (*i.e.* several months)

under mesocosm condition. It was also desired to be low cost, easy to set and maintain the desired water-table depth.

Such a controlled water-table plant growth system was established at the Open University field site in early spring 2003. The system was based on the methods of Snow and Tingey (1985), though certain modifications were made for use outdoors, including accounting for incoming precipitation. Effort was also made to supply water of equivalent quality to that of ground water.

3.2.2 Controlled water tension system: Set-up

The controlled water-table system is composed of three subsystems: a reservoir tank, a control float chamber and the mesocosms themselves. The system operates using a simple ball-valve, in which the water depth in the plant growing mesocosms and the control chambers equilibrate due to gravity.

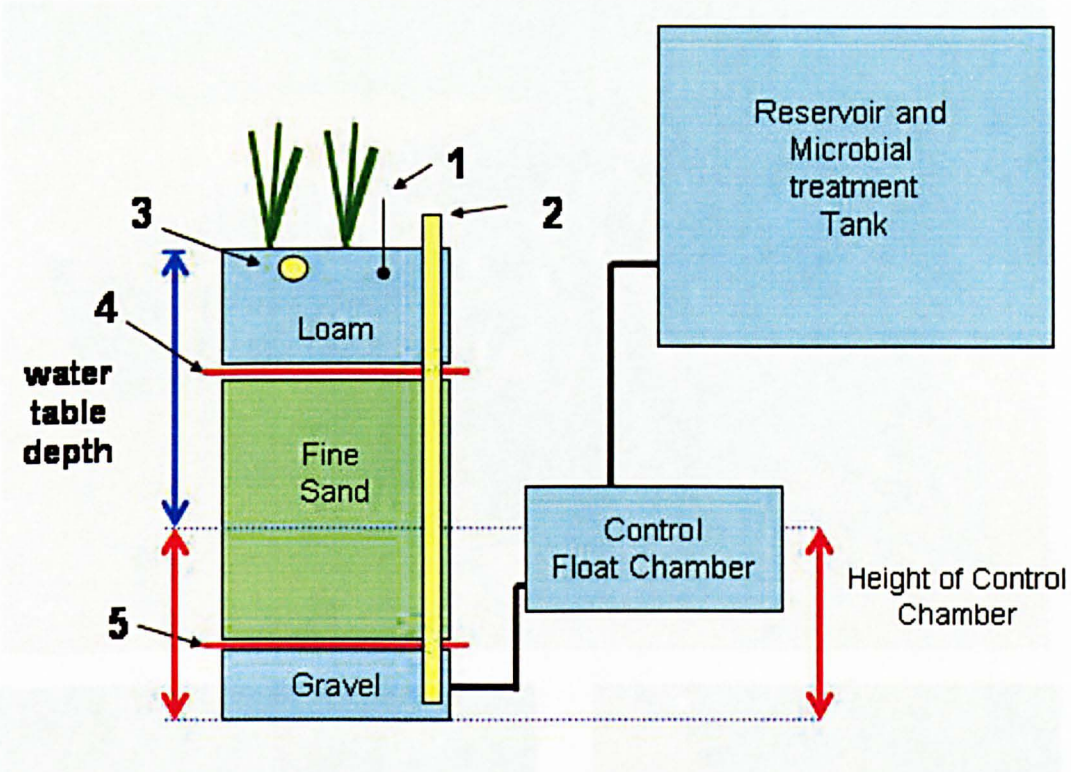


Figure 3.2a Schematic diagram of the controlled water-table system. The pots used were 55 cm high with 36 cm diameter (volume 50 litres). Description of the labels:

Label	Item	Description
1	Thermocouple	RS® Type T Thermocouple, with welded tip and PTFE insulation. Buried at 5 cm depth.
2	Dipwell	60 cm plastic tube, 2.5 cm diameter, holes every 5 cm. For direct measurement of water-table depth.
3	Ion-exchange resin bag	Amberlite® IR-120 and IR-900. Cation and anion exchange resin to measure nitrogen availability.
4	Root-exclusion fabric	Plastok® 52 μm nylon mesh. Prevents root penetration of fine sand.
5	Sand exclusion fabric	Weed control fabric. Prevents fine sand from seeping to the tubes.

Figure 3.2b The controlled water-table system overall view (top). Bottom photos show control chambers (a), a single mesocosm (b), and details of a single control chamber (c)

3.2.2.1 The reservoir tank

The water to the reservoir tank (capacity 1200 litres) was supplied from a local mains tap. This water was treated by submerging dried molassed sugar beet shreds (Trident Feeds ®, Peterborough) at 5 kg per month. This was done to deoxygenate and microbially remove any trace nitrate from the tap water, thus approximating ground water quality. Preliminary trials were made in the laboratory to identify the appropriate dosage and treatment time. Analysis of water samples with the dose mentioned above showed 90% reduction in dissolved oxygen (from 84% at inlet to 8% at the outlet) and the concentration of nitrate decreased from 15 ppm to < 1ppm.

3.2.2.2 The control float chamber

The control float chamber was composed of an 18 litre container, fitted with a ball and valve apparatus. The ball and valve apparatus regulated the flow of water from the reservoir tank into the chamber and subsequently into the mesocosms. The depth of the water level in the control chamber and its height aboveground was adjusted to match that of the desired level in the mesocosms using a total station device (T705, Leica Geosystems®, Switzerland). Water could flow freely in both directions between the float chamber and mesocosm. The chambers thus refilled with water from the reservoir tank to compensate for plant use in the mesocosms. When the mesocosm water-table depth rose as a result of precipitation, they lost water via overflow holes.

Five control float chambers were established to create water-table depths of 5, 15, 25, 35 and 45 cm below the soil surface in mesocosms. The control chambers were

connected by branching hose pipes (garden hose of diameter 12.5 mm) to the individual mesocosms.

3.2.2.3 The mesocosms

The containers were made of durable polyvinyl chloride with a height of 55 cm and diameter of 36 cm. Connection to the control float chamber was established with a pipe fitting at the base of the pots.

The pots were filled with multiple layers of gravel, sand and loam (See Figure 3.2a). The bottom 5 cm of the pot was filled with gravel to a level just above the inlet pipe connection. The gravel ensured a porous, non-clogging space where the incoming water could distribute itself freely. Weed control fabric was placed to separate this gravel from a 30 cm deep fine sand layer. This fine sand had an average particle size of 225 μm (WBB Minerals® RH65) and was placed to act as a conductive medium for water to the rooting medium. The loam-based rooting medium occupied the top 15 cm depth of the pot (at a density of 1.3 g cm^{-3}) and was prepared by mixing 1:1:2 proportions of peat moss, agricultural soil and sand. Furthermore, each pot was inoculated by 100 g of soil from a species-rich wet meadow, Cricklade North Meadow National Nature Reserve (National grid reference SU096958), to transfer existing microbial populations. The characteristics of the rooting medium and its soil water retention characteristics are given in Tables 3.1 and 3.2 respectively. Root penetration by plants beyond this loam and into the conductive fine sand medium was inhibited using 52 μm nylon mesh (Plastok® Birkenhead, UK), which was selected after testing a range of meshes between 30 and 175μm. Mesh size 52 μm effectively stopped plant

root growth while remaining porous to the passage of water. This choice also compares with similar mesh sizes used by other investigators namely 30 and 44 μm nylon mesh (Bethlenfalvay *et al.*, 1991; Kothari *et al.*, 1991). Furthermore the sides of the mesh were reinforced using tough black polythene to prevent side penetration by roots.

Table 3.1 Some physical and chemical characteristics of the soil used

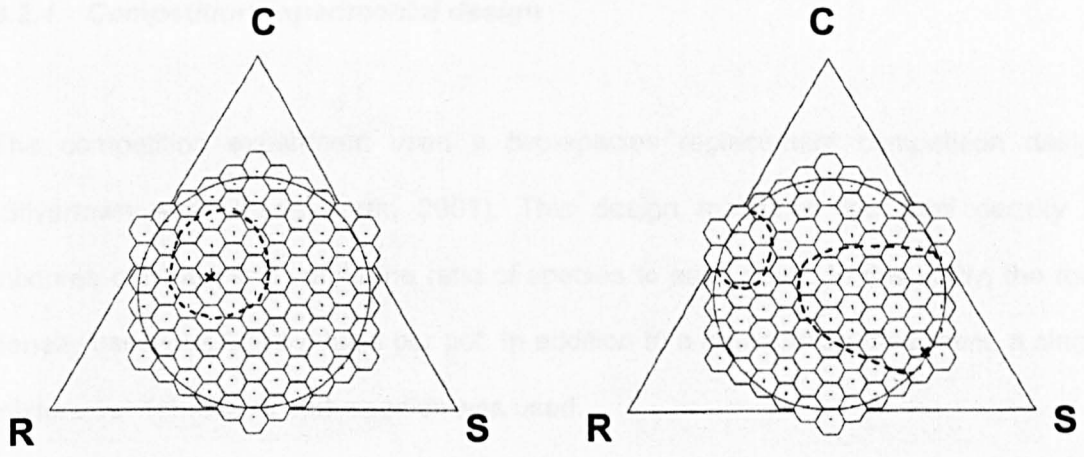
Soil Textural Class	***pH***	***C %***	***N %***	***C:N***	***Extractable K*** ($mg\ kg^{-1}$)	***Extractable P*** ($mg\ kg^{-1}$)	***Potential mineralizable N*** ($mg\ kg^{-1}$)
Loamy sand	8	2.1	0.11	19.1	65	65	22

Table 3.2 Soil water contents at selected water tensions

Soil Water Tension (cm)	**0**	**5**	**10**	**20**	**30**	**40**	**50**
Soil water content (% volume)	44	39	40	37	28	26	20
Air-Filled Porosity (% volume)	0	5	7	15	16	18	24

3.2.3 Plant materials

The plant species used in the mesocosm experiments were the meadow fescue *Festuca pratensis*, common sedge *Carex nigra* and greater burnet *Sanguisorba officinalis*. These plants represent three different categories: the meadow fescue is a grass (Poaceae), while the common sedge is a sedge (Cyperaceae) and the greater burnet a forb (Rosaceae). These three species were selected as they have been observed to coexist in the field, though with specific preferences to water-regime (See section 1.3 in Chapter 1). Moreover, these species also show differing life strategies according to Grime's Competitor - Stress tolerator - Ruderal (CSR) triangular ordination (Grime *et. al.*, 1988). An example is shown in Figure 3.3.



Festuca pratensis *Carex nigra*

Figure 3.3 Grime's C-S-R triangular ordination life strategy for the meadow fescue *Festuca pratensis* and common sedge *Carex nigra*

The strategy of *F. pratensis* appears that of competitive but also ruderal nature, (CR) while that of *C. nigra* is of tolerance to stress.

Parent plants of the species were collected from Cricklade North meadow national nature reserve and asexually multiplied by splitting in the greenhouse. These plants were kept for a year to mature before being used for the study. Cloned material was used to ensure uniformity. A number of separate clones were selected to improve representativity. The use of clonal materials is well known in experimental, environmental and competition investigations where minimum genetic variation is required (e.g. Antonovics, 1987; Wijesinghe and Hutchings, 1997). This was of vital importance for this study as the primary aim was to investigate subtle variations of an environmental gradient.

3.2.4 Competition experimental design

The competition experiment used a two-species replacement competition design (Silvertown and Charlesworth, 2001). This design maintains the *total* density of mixtures constant and varies the ratio of species to each other. In this study, the total density used was 6 individuals per pot. In addition to a monoculture treatment, a single mixture *i.e.* 1:1 ratio of both species was used.

For the main experiment, the meadow fescue *F. pratensis* and the common sedge *C. nigra* were used. Three plant combinations, *i.e.* two monocultures and a mixture, were then replicated in four blocks for five water level treatments, resulting in 60 experimental pots. The pots were arranged randomly within the four blocks. In this experiment for each mesocosm, 6 individuals of either *Festuca pratensis* and *Carex nigra* in monocultures or 3 of each in mixture were planted. The five water-table depth treatments used were 5, 15, 25, 35 and 45 cm.

Meanwhile an outer row of 36 pots were prepared using *C. nigra* and the greater burnet, *S. officinalis* along similar conditions except using only two water-table depth treatments *i.e.* 5 and 45 cm. These were not harvested in the first season of 2003 but subjected to fertilization experiment in 2004. For this a total fertiliser amount equivalent to 56 kg ha^{-1} of ammonium nitrate (NH_4NO_3) was applied in split ratio of every 6 days during the growing season. This amount is smaller than usually is applied in agricultural pastures, but it has been associated with significant change in plant community composition (Mountford *et al.*, 1993). This experiment was established to see whether fertilization negates the effect of water-table depth on plant competition. Although the original plan was to use *C. nigra* versus *F. pratensis*, due to lack of sufficient clonal

materials, *S. officinalis* was selected as an alternative. However it may be seen from the Ellenberg values and also later confirmed from our field observations, *S. officinalis*, even though a forb, offers a similar contrast to *C. nigra* and hence is a suitable replacement.

	Block α	**Block β**	**Block γ**	**Block δ**	
	A2-f	*E3-u*	*A1-u*	*A3-f*	
A3-u	C3	D1	D1	A3	
E3-f	B1	E3	E2	D1	*A1-f*
E2-f	A2	D3	E3	C3	*E1-f*
E1-u	D3	C1	D3	A1	*E1-u*
A1-f	E3	B2	B1	E2	*A-2-u*
E1-f	B2	C2	A1	D2	*A1-u*
A3-f	E2	A1	B3	B3	*E2-u*
E1-u	C1	B1	C2	C2	*E3-f*
A1-u	E1	D2	A2	D3	*E2-f*
A2-u	B3	A2	C1	B2	*A3-u*
E3-u	D2	C3	C3	E1	*E1-f*
E3-f	A1	E1	D2	C1	*A1-f*
E2-f	C2	E2	B2	E3	*A3-f*
A2-f	D1	B3	A3	B1	*A3-u*
	A3	A3	E1	A2	*E3-u*
	E2-u	*A2-u*	*E2-u*	*A2-f*	

Figure 3.4 Diagram of the experimental layout. A, B, C, D, E are water treatments 5, 15, 25, 35, 45 cm respectively. Unshaded rows represent the main experiment. 1 (*F. pratensis*), 2 (*C. nigra*), 3 (Mixture) are species combinations. Shaded rows were used for fertilization experiment: 1 (*S. officinalis*), 2 (*C. nigra*) 3 (Mixture); -f (fertilized) -u (unfertilized)

Modified Long Ashton nutrient solution (Hewitt, 1952) was added to the mesocosm plants in their second season of growth in spring 2004. This nitrogen-free solution was applied at full strength at a dose of 1 litre per pot every fortnight between April 11th and June 5th 2004. The application dose was made at this level to supply small amounts at frequent intervals and therefore avoid leaching loss.

The need for applying nutrient solution was necessary to replace the harvest off take during the first growing season.

3.2.4.1 Data collection

The mesocosm system was assessed for reliability by examining the achieved soil water tensions. The outcome of competition was studied from measures on plant cover, dry matter production and tissue nutrient content. Details of these are given as follows.

Measurement of soil water levels

The soil moisture status was monitored both through dipwells buried in the mesocosm and tensiometers in the rooting medium (type SWT3, Delta-T® Devices Ltd, Cambridge, UK). More subtle differences in daily water level fluctuation were monitored using automatic logging pressure transducers (Eijkelkamp® Divers, The Netherlands) suspended in the dipwells. The difference in pressure between that of a submerged transducer and a second transducer at atmospheric pressure tracked changes in water-table depth.

The system was assessed for 1) the relation of dipwell readings of water-table depth to tensiometer readings, 2) the comparison between expected water table depths and measured water table depths in the mesocosms, 3) the maintenance of water table depth over the duration of the experimental period, and 4) the response of the system to peaks of high evaporative demand and also that of rainstorms.

Plant cover and dry matter production

Plant cover was estimated using a pin-quadrat. The pin-quadrat was considered appropriate to measure relative cover as it could be rotated from a central axis. A record was made of the species touched by each drop of the pin through to the soil surface (Chalmers and Parker, 1989). Four transects were done for each mesocosm and a sum of results made for analysis. Cover was then compared between ratios of monoculture versus mixture, and between both species.

The aboveground plant biomass production was assessed by harvesting at 2 cm height above the soil surface on 27th June, which coincides with the traditional hay cut. The harvested plant matter was dried at 55 °C for 72 hours before weighing. Plant roots were sampled by taking a core, 5 cm diameter and 10 cm deep (volume 196 cm^3). Two cores were taken for the monoculture treatment and 3 cores for mixture pots. Then the plant roots were washed out and dried in a similar fashion as the aboveground matter before being weighed.

Plant material analysis

The harvested and dried plant material was ground finely for nutrient analysis. For carbon and nitrogen, the analysis was done using LECO 2000®Elemental analyser (LECO® St. Joseph, Michigan, USA). The method involves rapid combustion of 0.2 g of plant sample with a an accelerator flux (COMCAT® LECO) and analysis of the gases produced.

For plant tissue phosphorus analysis, 0.5 g of ground plant material was combusted at 450 °C in a muffle furnace. The ash was then dissolved in 5 ml of 2 M HCl which was then made up to 50 ml with deionized water (Ryan *et al.*, 2001). This was then mixed with Barton's colour reagent before analysing by Helios® Thermo Spectroscopic colorimeter at a wavelength of 410 nm (MAFF, 1986). For analysing plant tissue potassium, the ashed and dissolved plant matter was passed through Gallenkamp SGA_330C® flame photometer (MAFF, 1986).

3.2.4.2 Analysis of competition

Plant competition was analysed from the data on dry matter production and nutrient concentration along the five levels of water availability. The replacement competition design was assessed using the relative yield formula of de Wit and van den Bergh (1965) (cited in Wilson, 1988) as follows:

$$\text{Relative yield of species 'i'} = \frac{\text{yield of species 'i' in mixture}}{\text{Yield of species 'i' in monoculture}}$$

$$\text{Relative yield of species 'j'} = \frac{\text{yield of species 'j' in mixture}}{\text{Yield of species 'j' in monoculture}}$$

The total biomass produced both aboveground and belowground was combined for the general analysis. However, to study plant resource allocation to belowground and aboveground parts, root to shoot ratio was used. Data were initially tested for normality and no transformation was necessary. Statistical analyses e.g. analysis of variance and t-test were made using Statistica® 6.0 package. Significant analyses of variance ($p < 0.05$) were further ranked using Tukey HSD test.

3.3 Results

3.3.1 Controlled water tension system

Although there were only five control float chambers put at five different heights to feed 12 mesocosm each, due to the undulating nature of the ground and subsequent subsidence of the soil, a range of water levels occured. However t-test on the expected and observed water-table depths found that the achieved levels were similar ($p = 0.05$). See Table 3.3. The achieved water-table depth and measured water tension also correlate significantly ($r^2 = 0.98$).

Table 3.3 Comparison of calculated and observed water-table depths. Mean and standard deviation are shown.

Water-table depth Treatment (cm)	Calculated Water-table depth (cm)	Measured Water-table depth (cm)
5	5.16 ± 2.52	6.00 ± 2.50
15	15.22 ± 2.40	13.54 ± 2.73
25	25.11 ± 1.95	25.4 ± 2.31
35	34.73 ± 2.09	33.15 ± 1.40
45	44.56 ± 2.58	42.19 ± 2.39

Maintenance of the water-table depth during the experimental period was monitored using pressure-transducers and tensiometers for fine scale variations and dipwells over the long term. Pressure transducer readings made every five minutes over the summer period showed that the water-table depths could be maintained with little variation, at most ± 2 cm, over the course of several weeks, even during high evapotranspiration periods. An example of this over a one week period in summer is shown in Figure 3.5.

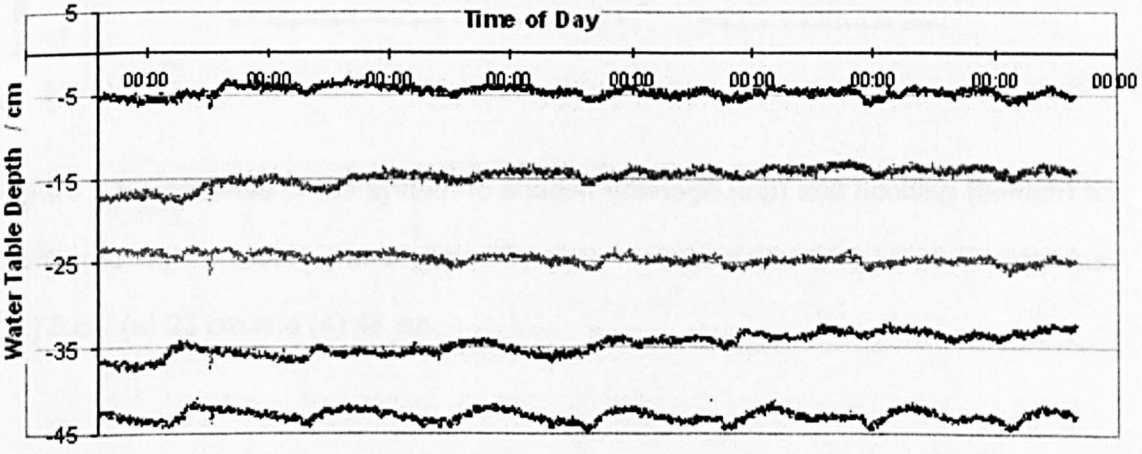


Figure 3.5 Pressure transducer readings on the maintenance of water-table depth. A sample week between 10/8/03 – 17/8/03 is shown. Readings were made every 5 minutes.

The response of the system to peaks of high evaporative demand and that of rain storms was tested by events of sudden drainage and flooding. Sensitive pressure transducer readings showed it was possible to restore the water-regime within 20 minutes of the events (Figure 3.6).

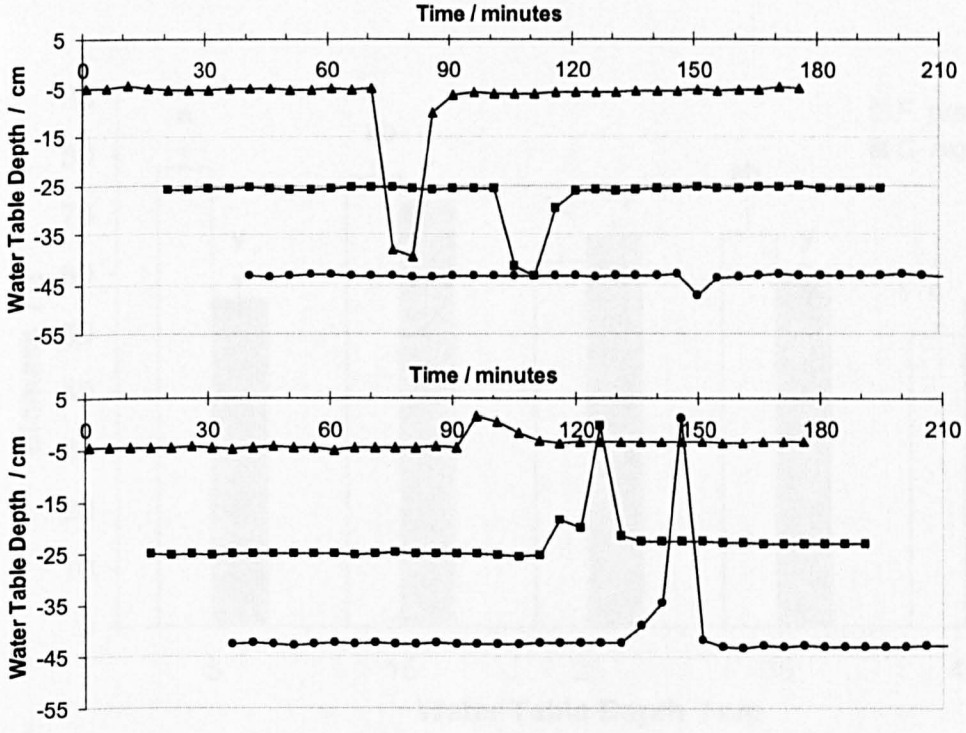


Figure 3.6 Response of the system to sudden drainage (top) and flooding (bottom) as monitored with pressure transducers. Symbols denote water-table depth treatments: (▲) 5 cm (■) 25 cm and (●) 45 cm.

3.3.2 Plant biomass production and resource allocation

3.3.2.1 Plant biomass production response to water-regime: case of monocultures

Total biomass production of *F. pratensis* and *C. nigra* in monoculture during the experiment is given in Figure 3.7.

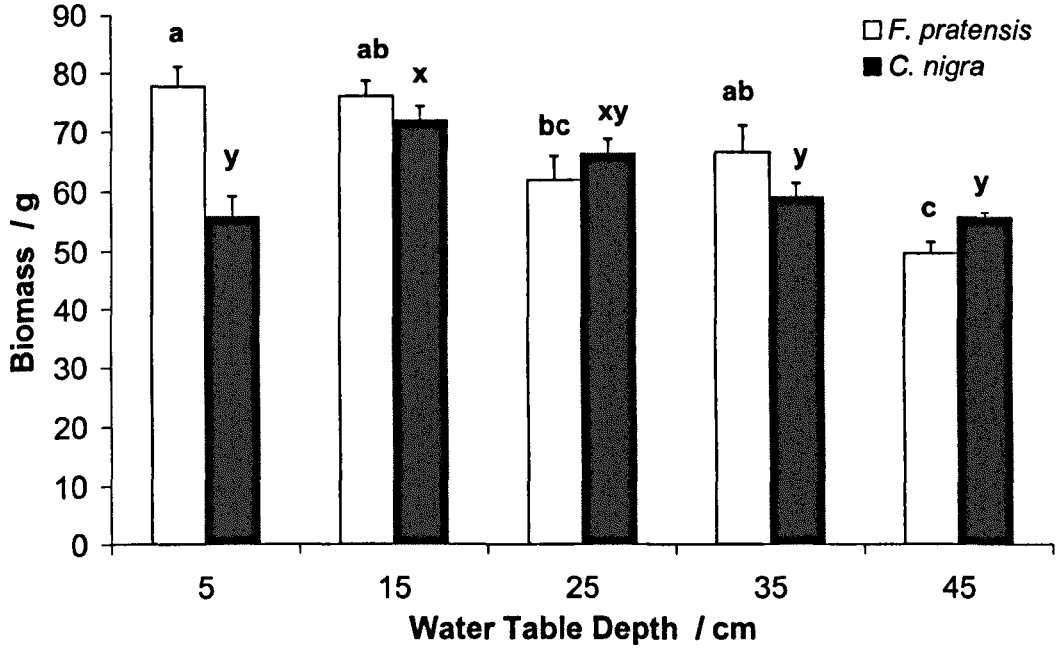


Figure 3.7 Total biomass production in monoculture of *F. pratensis* and *C. nigra* in response to water-table depth. Post-hoc Tukey ranking at p = 0.05, is indicated by a, b, c for *F. pratensis* and x, y for *C. nigra*. Bars show standard error.

Figure 3.7 shows the overall response of the two species was not different, with both producing highest dry matter production at the wetter end (water-table depth of < 15 cm) with the lowest at the driest end (water-table depth of 45 cm).

Plant biomass production for *F. pratensis* and *C. nigra* in monoculture varied significantly ($p < 0.001$) along the water-table depth. The analysis of variance tables for these are given in Table 3.4 a and b.

Table 3.4 Analysis of Variance Table for biomass production in monoculture (a) *F. pratensis* (b) *C. nigra*

(a) Effect	*SS*	*df*	*MS*	*F*	*p*
Water-regime	2100.86	4	525.21	11.51	< 0.001
Error	684.44	15	45.63		

(b) Effect	*SS*	*df*	*MS*	*F*	*p*
Water-regime	810.90	4	202.72	8.42	< 0.001
Error	361.24	15	24.08		

3.3.2.2 Plant biomass production response to water-regime: case of mixtures

Plant biomass production response to different water-table depths in mixture is given in Figure 3.8.

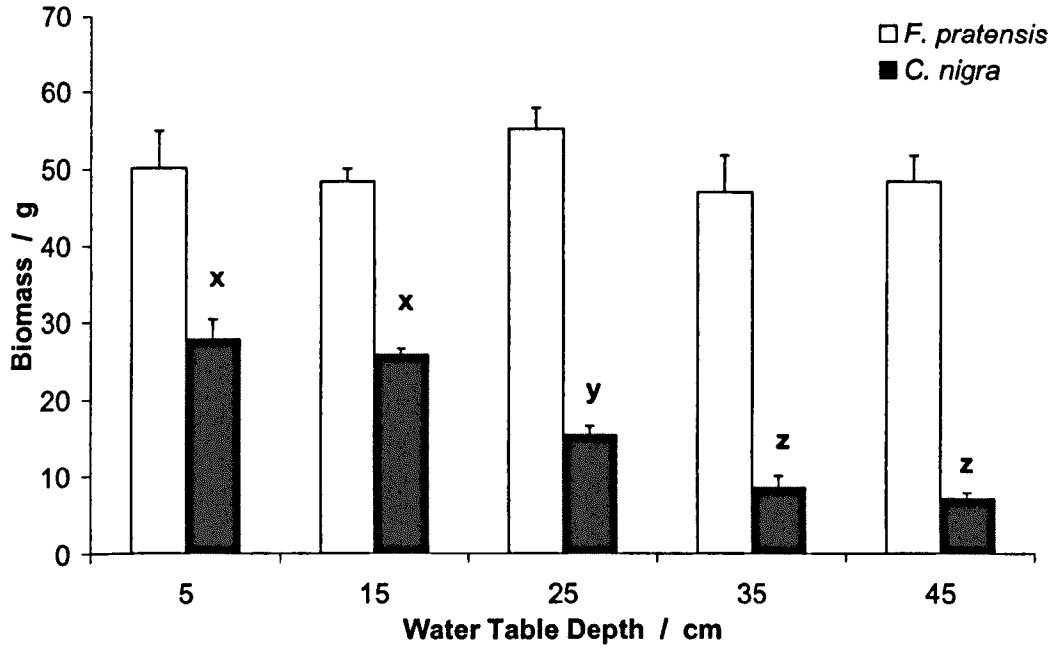


Figure 3.8 Total biomass production in mixture of *F. pratensis* and *C. nigra* in response to water-table depth. The letters x, y and z indicate post-hoc Tukey ranking at p=0.05 for *C. nigra*. Bars show standard error.

Plant biomass production for *F. pratensis* along the water-table depth did not show a significant difference. However *C. nigra* showed a very marked response to water-table depth ($p < 0.001$). The analysis of variance for both species is shown in Table 3.5a and b.

Table 3.5 Analysis of Variance Table for biomass production in mixture (a) *F. pratensis* (b) *C. nigra*

(a) Effect	*SS*	*df*	*MS*	*F*	*p*
Water-regime	158.65	4	39.66	0.75	0.571
Error	789.94	15	52.66		

(b) Effect	*SS*	*df*	*MS*	*F*	*p*
Water-regime	1463.44	4	365.86	40.01	< 0.001
Error	137.18	15	9.15		

The biomass production in mixture shows a more pronounced difference in the response between the two species. Biomass production of *F. pratensis* maintains its monoculture production in the drier treatments. However, for *C. nigra* it shows a sharp decline with soil drying when grown in mixture compared to monoculture. This is demonstrated by examination of the relative yield (Figure 3.9).

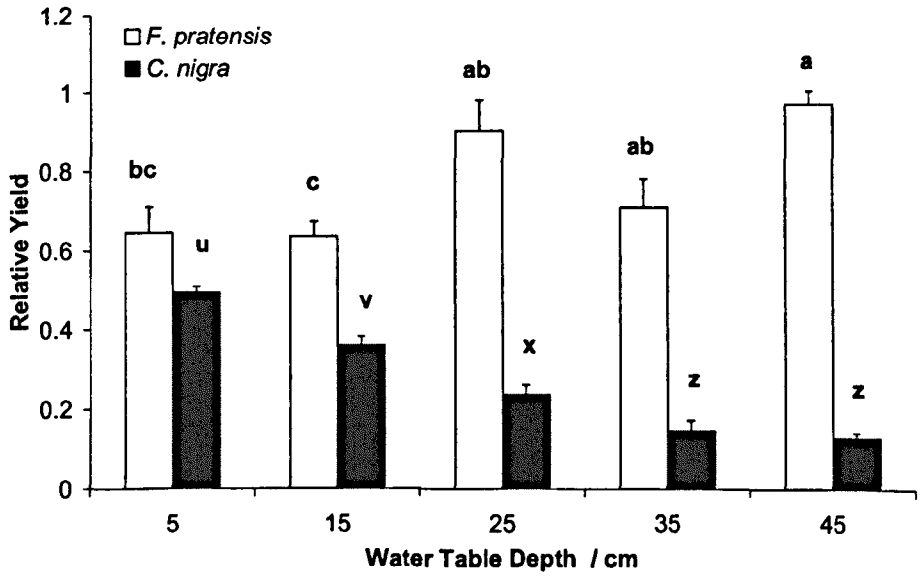


Figure 3.9 Relative Yield (ratio of yield in mixture to monoculture) for *F. pratensis* and *C. nigra* in response to water-table depth. Post-hoc Tukey ranking at p = 0.05, is indicated by a, b, c for *F. pratensis* and u, v, x, z for *C. nigra*. Bars show standard error.

The analysis of variance for relative yield of *F. pratensis* and *C. nigra* is given in Table 3.6. The results reveal significant influence of water-regime on the response of both species.

Table 3.6 Analysis of Variance Table for Relative Yield of (a) *F. pratensis* (b) *C. nigra*

(a) Effect	*SS*	*df*	*MS*	*F*	*p*
Water-regime	0.378	4	0.094	6.55	< 0.01
Error	0.216	15	0.014		

(b) Effect	*SS*	*df*	*MS*	*F*	*p*
Water-regime	0.385	4	0.096	57.40	< 0.001
Error	0.025	15	0.002		

3.3.2.3 Biomass allocation in response to water-regime: Root to Shoot Ratio

Biomass allocation, in terms of root to shoot ratio, by *F. pratensis* and *C. nigra* in response to varying water-table depths in monoculture is shown in Figure 3.10.

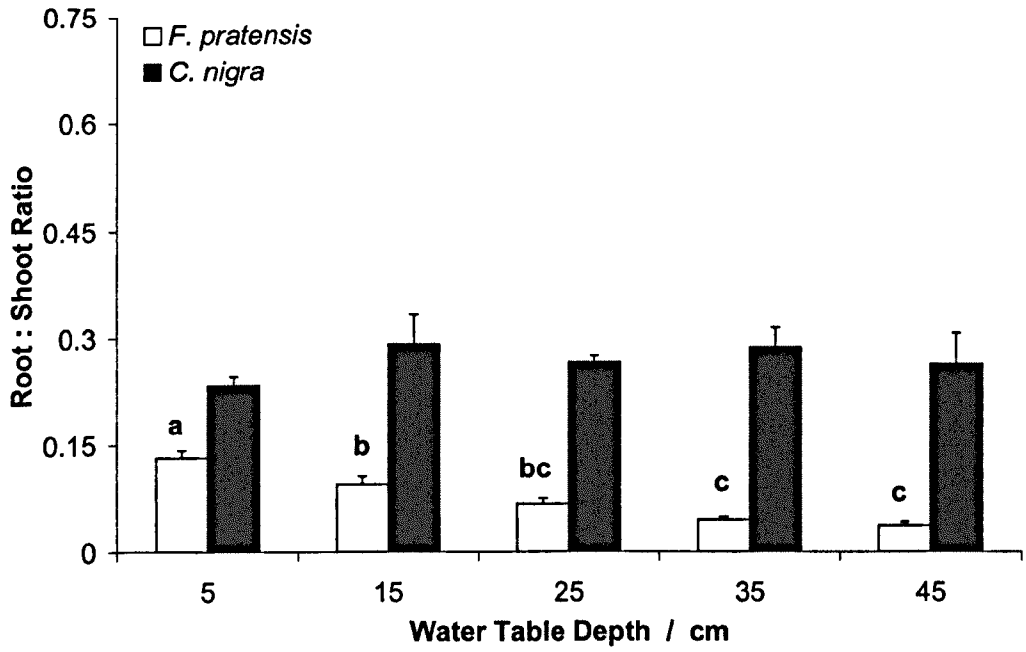


Figure 3.10 Biomass allocation (root to shoot ratio) of *F. pratensis* and *C. nigra* in response to water-table depth in monoculture. Post-hoc Tukey ranking at p = 0.05, is indicated by a, b, c for *F. pratensis*. Bars show standard error.

Root to shoot ratio in monoculture significantly decreased with soil drying for *F. pratensis*. However, for *C. nigra* it did not respond significantly to water-table depth. The results of the analysis of variance performed for the root to shoot ratio is given in Table 3.7.

Table 3.7 Analysis of variance table for root to shoot ratio in monoculture for (a) *F. pratensis* (b) *C. nigra*

(a) Effect	*SS*	*df*	*MS*	*F*	*p*
Water-regime	0.024	4	0.006	22.26	< 0.001
Error	0.00405	15	0.00027		

(b) Effect	*SS*	*df*	*MS*	*F*	*p*
Water-regime	0.00854	4	0.00214	0.585	0.68
Error	0.0547	15	0.00365		

Biomass allocation, in terms of root to shoot ratio, by *F. pratensis* and *C. nigra* in response to varying water-table depths in mixture is shown in Figure 3.11.

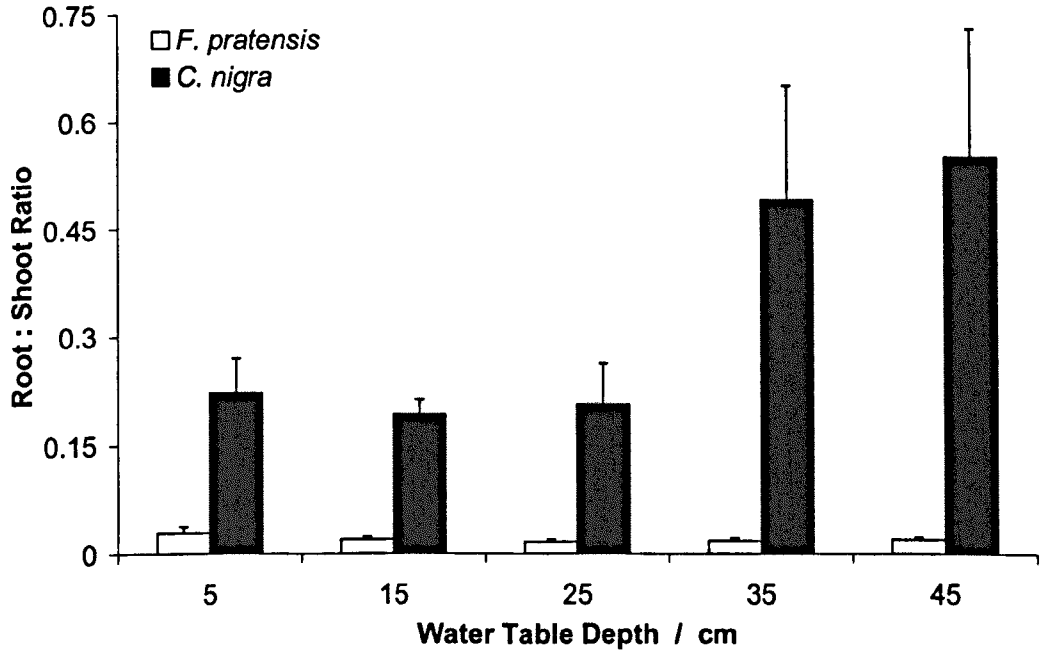


Figure 3.11 Biomass allocation (root to shoot ratio) of *F. pratensis* and *C. nigra* in response to water-table depth in mixture. Bars show standard error.

The analysis of variance for root to shoot ratio of *F. pratensis* and *C. nigra* in mixture did not show any significant response to water-table depth. See Table 3.8.

Table 3.8 Analysis of variance table for root to shoot ratio in mixture (a) *F. pratensis* (b) *C. nigra*

(a) Effect	*SS*	*df*	*MS*	*F*	*p*
Water-regime	0.00033	4	0.000083	0.941	0.47
Error	0.00132	15	0.000088		

(b) Effect	*SS*	*df*	*MS*	*F*	*p*
Water-regime	0.48270	4	0.1206	2.420	0.094
Error	0.74786	15	0.0499		

3.3.3 Plant tissue nutrient concentrations

Response of aboveground plant tissue nutrient concentrations *i.e.* nitrogen, phosphorus and potassium, to differences in water-table depth were also assessed. The results for each species are shown in Figures 3.12 - 3.14.

Tissue nitrogen concentration in monoculture for *F. pratensis* and *C. nigra* is given in Figure 3.12a.

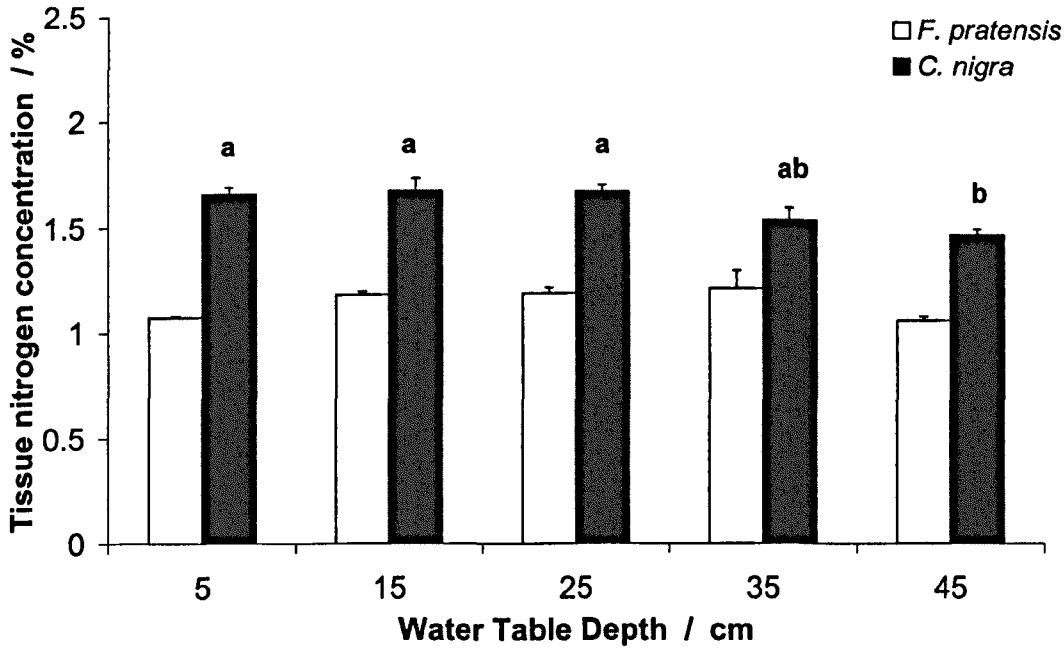


Figure 3.12a Tissue nitrogen concentration of *F. pratensis* and *C. nigra* plants in monoculture. Letters a, b and c represent Tukey post-hoc rankings at p = 0.05. Bars represent standard error.

Analysis of variance in monoculture showed significant effect of water-regime on tissue nitrogen concentration of *C. nigra* ($p < 0.05$). A marginally significant response of tissue nitrogen concentration to water-table depth was also found for *F. pratensis* ($p = 0.054$). See Table 3.9.

Table 3.9 Analysis of variance for tissue nitrogen concentration of *F. pratensis* and *C. nigra* in monoculture

(a) Effect	*SS*	*df*	*MS*	*F*	*p*
Water-regime	0.08018	4	0.02005	2.974	0.054
Error	0.10111	15	0.00674		

(b) Effect	*SS*	*df*	*MS*	*F*	*p*
Water-regime	0.12943	4	0.0324	4.75	0.014
Error	0.08861	13	0.0068		

On the other hand, in mixture (Figure 3.12b), there was significant effect of water-table depth on *C. nigra* response ($p < 0.001$). Once again, for *F. pratensis* the response was significant only marginally ($p < 0.073$). See Table 3.10.

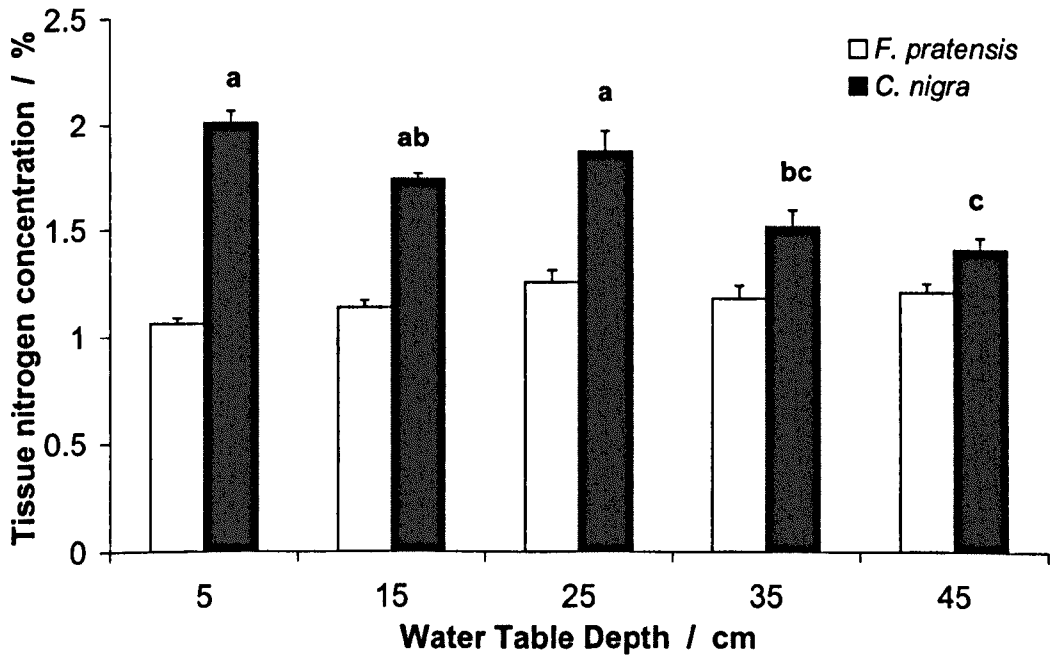


Figure 3.12b Tissue nitrogen concentration of *F. pratensis* and *C. nigra* plants in mixture. Letters a, b and c represent Tukey post-hoc rankings at p = 0.05. Bars represent standard error.

Table 3.10 Analysis of variance for tissue nitrogen concentration of (a) *F. pratensis* and (b) *C. nigra* in mixture

(a) Effect	*SS*	*df*	*MS*	*F*	*p*
Water-regime	0.0851	4	0.0213	2.67	0.073
Error	0.120	15	0.00798		

(b) Effect	*SS*	*df*	*MS*	*F*	*p*
Water-regime	0.991	4	0.248	13.17	<0.001
Error	0.282	15	0.0188		

The tissue phosphorus concentration for *F. pratensis* and *C. nigra* is given in Figures 3.13 a and b.

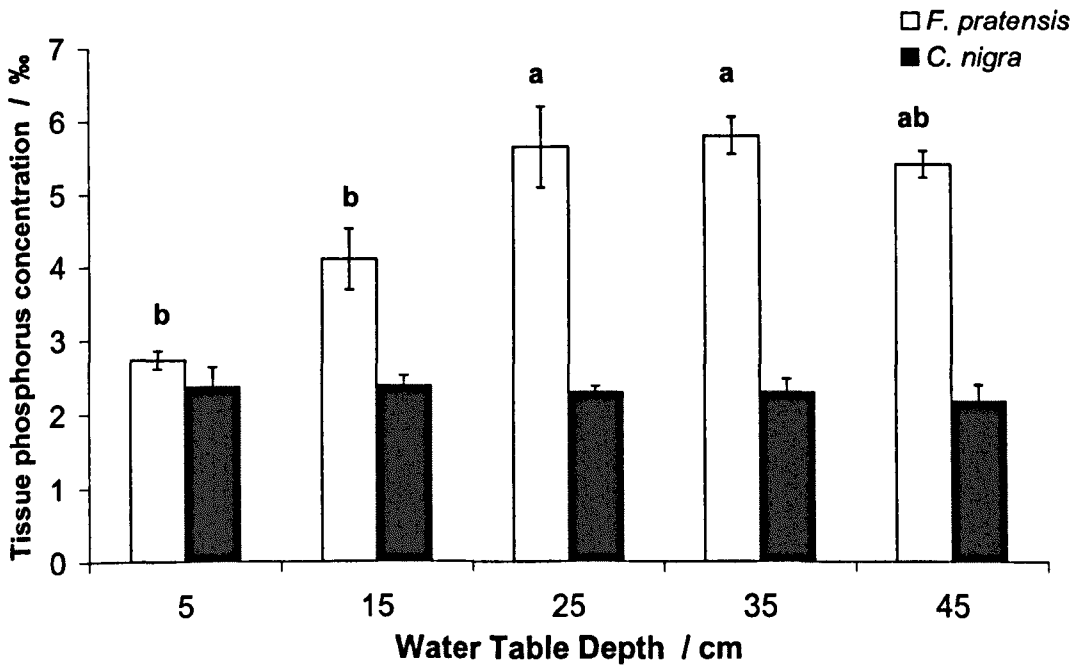


Figure 3.13a Tissue phosphorus concentration of *F. pratensis* and *C. nigra* plants in monoculture. Letters a and b represent Tukey post-hoc rankings at p = 0.05. Bars represent standard error.

The above figure shows tissue phosphorus concentration in monoculture showed significant influence of water-table depth on *F. pratensis* ($p < 0.001$) but not for *C. nigra* (See Table 3.11).

Table 3.11 Analysis of variance for tissue phosphorus concentration of *F. pratensis* (a), and *C. nigra* (b) in monoculture

(a) Effect	*SS*	*df*	*MS*	*F*	*p*
Water-regime	19.936	4	4.984	13.29	< 0.001
Error	3.374	9	0.375		

(b) Effect	*SS*	*df*	*MS*	*F*	*p*
Water-regime	0.0978	4	0.0244	0.2170	0.92
Error	1.352	12	0.1126		

However in mixture, both *C. nigra* and *F. pratensis* showed a significant response (Figure 3.13b). The analysis of variance tables for this are given in Table 3.12.

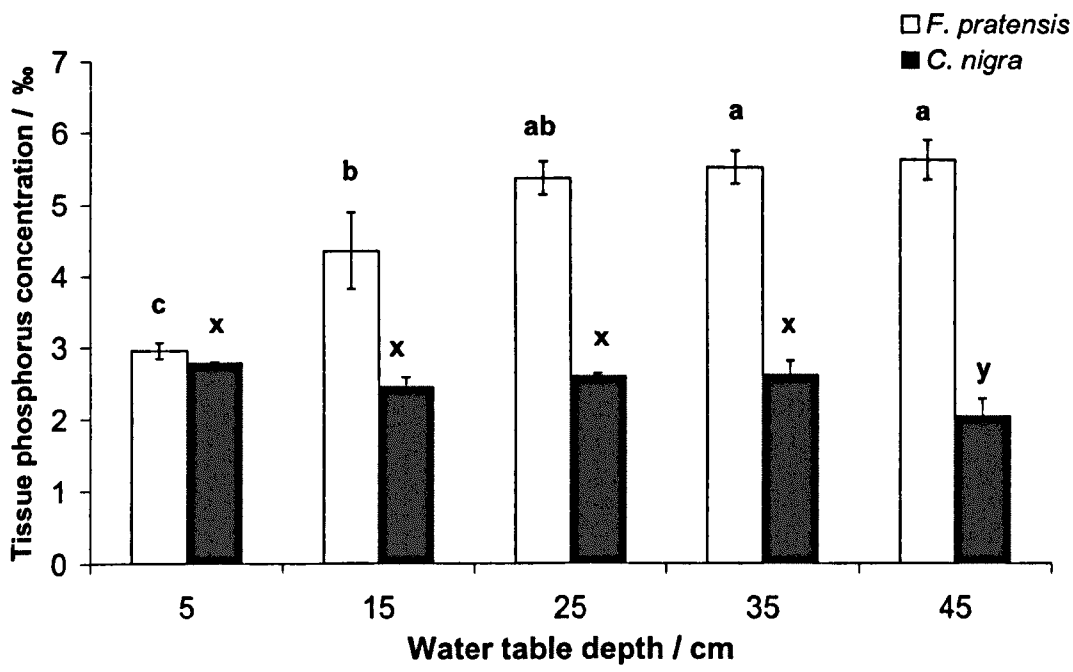


Figure 3.13b Tissue phosphorus concentration of *F. pratensis* and *C. nigra* plants in mixture. Letters a, b, c and x, y represent Tukey post-hoc rankings at p = 0.05 for *F. pratensis* and *C. nigra* respectively. Bars represent standard error.

Table 3.12 Analysis of variance for tissue phosphorus concentration of *F. pratensis* (a), and *C. nigra* (b) in mixture

(a) Effect	*SS*	*df*	*MS*	*F*	*p*
Water-regime	20.207	4	5.052	17.85	<0.001
Error	3.963	14	0.283		

(b) Effect	*SS*	*df*	*MS*	*F*	*p*
Water-regime	0.763	4	0.191	4.48	0.025
Error	0.426	10	0.0426		

Plant tissue potassium concentration of *F. pratensis* and *C. nigra* response to water-table depth is given in Figure 3.14 a and b.

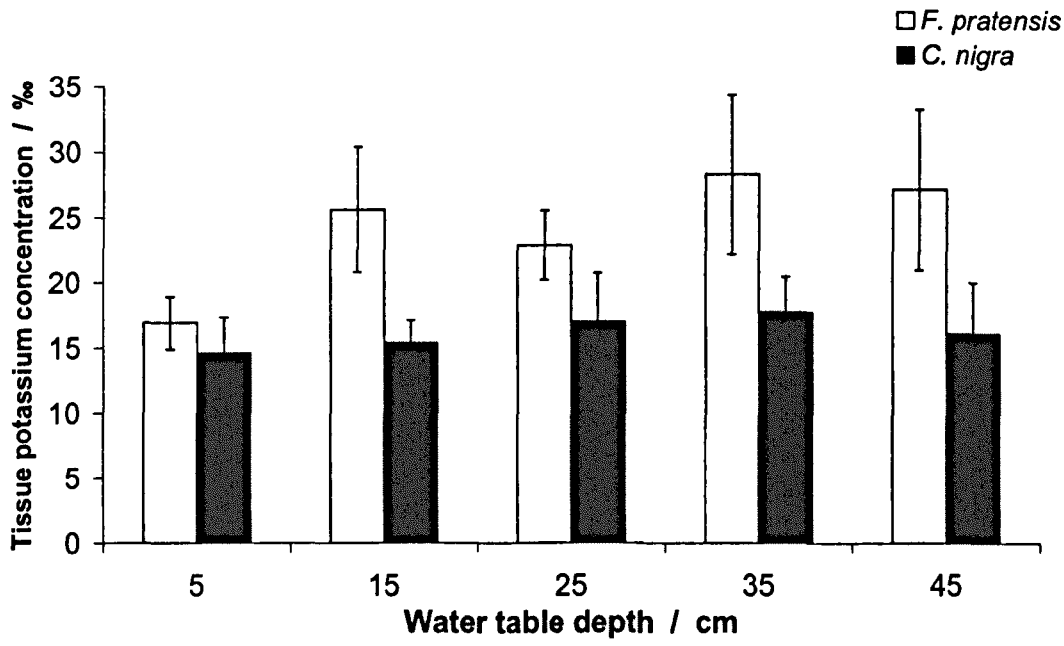


Figure 3.14a Tissue potassium concentration of *F. pratensis* and *C. nigra* plants in monoculture. Bars represent standard error.

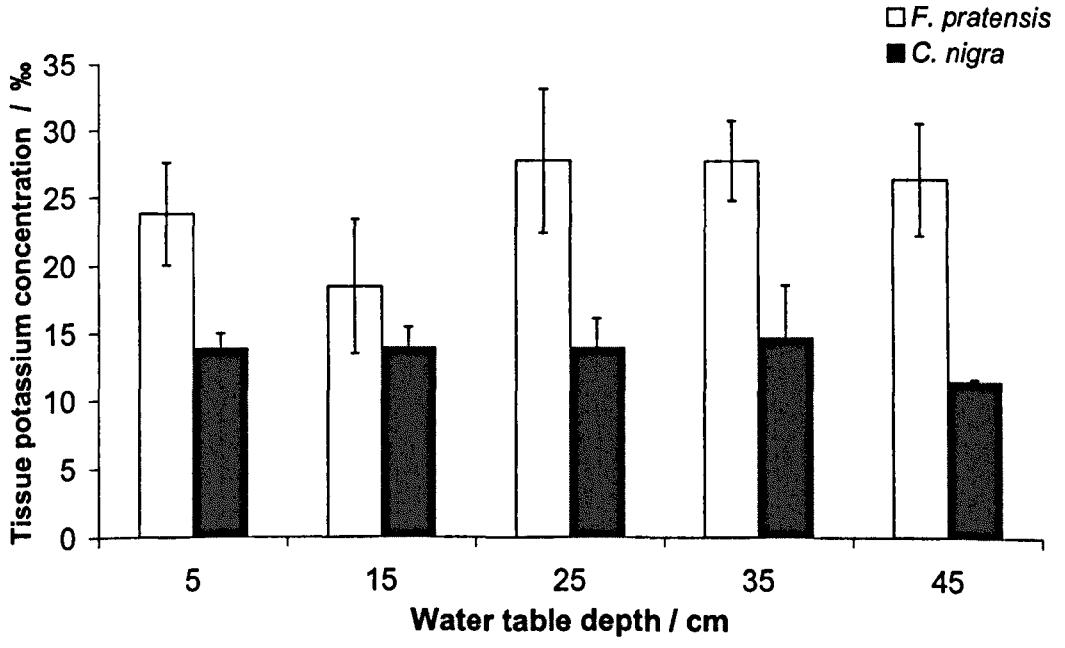


Figure 3.14b Tissue potassium concentration of *F. pratensis* and *C. nigra* plants in mixture. Bars represent standard error.

The analysis of variance for tissue potassium concentration showed there was no significant effect of water-regime on tissue potassium concentration in both monoculture and mixture.

3.3.4 Effect of nitrogen fertilization and water-regime

The response of aboveground biomass production by *S. officinalis* and *C. nigra* to nitrogen fertilization is shown in Figure 3.15 and 3.16.

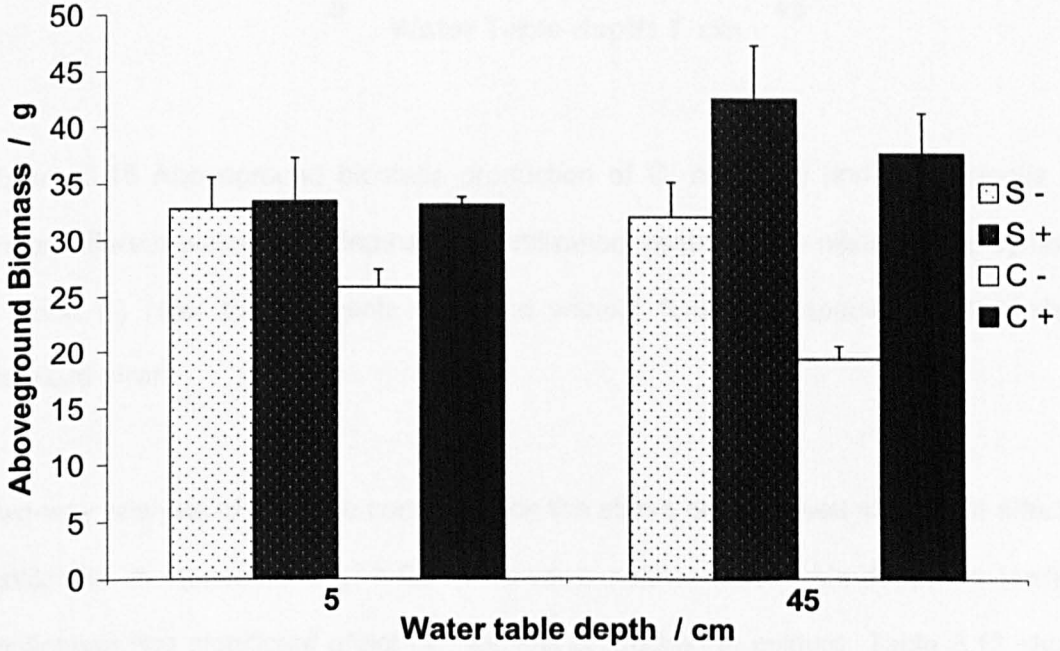


Figure 3.15 Aboveground biomass production of *C. nigra* (C) and *S. officinalis* (S) under different water-table depths and fertilization treatments in monoculture. The symbols (+) and (-) refer to treatments with and without fertilizer respectively. Bars show standard error.

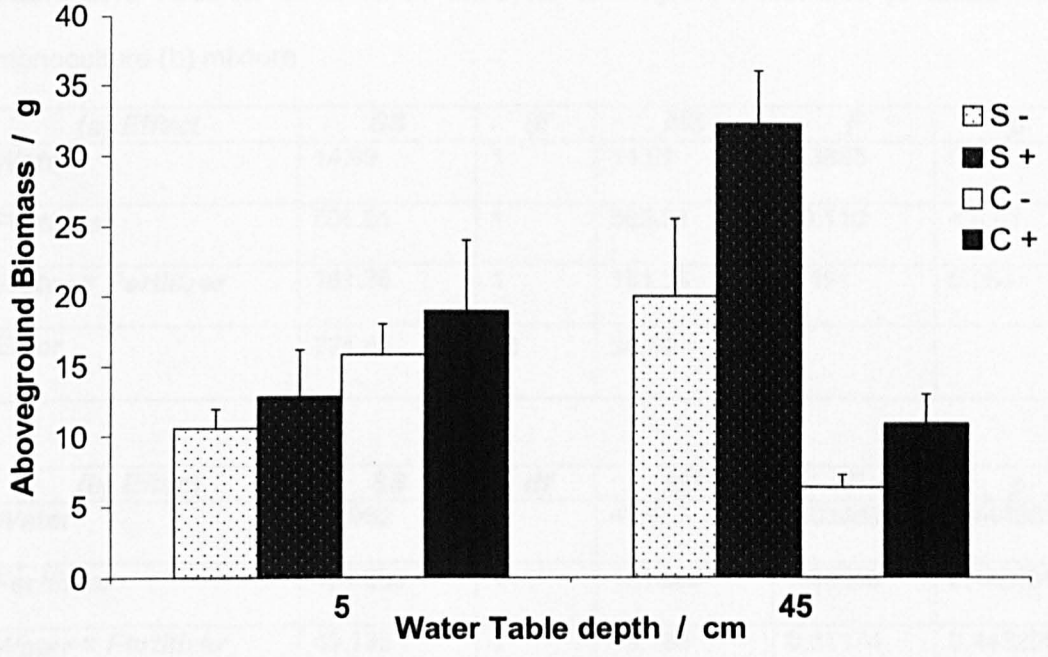


Figure 3.16 Aboveground biomass production of *C. nigra* (C) and *S. officinalis* (S) under different water-table depths and fertilization treatments in mixture. The symbols (+) and (-) refer to treatments with and without fertilizer respectively. Bars show standard error.

Two-way analysis of variance conducted on the above data showed significant effect of fertilization in monoculture ($p < 0.01$). However neither water-table depth nor fertilizer application had significant effect on biomass production in mixture. Table 3.13 shows these values.

Table 3.13 Analysis of variance table for aboveground biomass production in (a) monoculture (b) mixture

(a) Effect	*SS*	*df*	*MS*	*F*	*p*
Water	14.99	1	14.99	0.3885	0.54
Fertilizer	505.91	1	505.91	13.110	< 0.01
Water × Fertilizer	161.36	1	161.36	4.181	0.054
Error	771.82	20	38.59		

(b) Effect	*SS*	*df*	*MS*	*F*	*p*
Water	48.963	1	48.963	0.60960	0.444081
Fertilizer	181.830	1	181.830	2.26383	0.148056
Water × Fertilizer	49.135	1	49.135	0.61174	0.443296
Error	1606.397	20	80.320		

Following the above two-way analyses of variance, individual one-way analyses of variance were conducted to determine the single effects of water-table depth on each species with and without fertilization. The analyses of variance results on biomass production and tissue nitrogen content are summarized in Table 3.14. The raw data and the individual analyses of variance are given in Appendix 3.

Table 3.14 Significance of one-way analyses of variance (p = 0.05) results of the influence of water-regime on plant response on: (a) biomass production and (b) tissue nitrogen concentration. Significant, p values are given; 'ns' means not significant

a) Biomass	*S. officinalis*		*C. nigra*	
	Unfertilized	**Fertilized**	**Unfertilized**	**Fertilized**
Monoculture	ns	ns	p = 0.03	ns
Mixture	p = 0.02	p = 0.02	p = 0.02	ns

b) Tissue Nitrogen	*S. officinalis*		*C. nigra*	
	Unfertilized	**Fertilized**	**Unfertilized**	**Fertilized**
Monoculture	p = 0.04	ns	ns	ns
Mixture	p = 0.01	ns	ns	ns

The shaded regions in above table show that the dependence of biomass production on water-table depth for *C. nigra*, seen under unfertilized conditions is turned off when fertilization is made. On the other hand, a similar switch is observed with tissue nitrogen concentration for *S. officinalis*. *S. officinalis* tissue concentration of nitrogen is dependent on water-table depth without fertilization, however under fertilization this dependence disappears.

3.4 Discussion

Competition processes are modelled by referring to the fundamental niche of the species which is observed by growing it in monoculture. Observation of its response in multi-species mixtures gives indications of the realized niche. The relative performance

of the species in these two cases is then compared to understand the effect of competition (Austin, 1982).

3.4.1 Plant biomass production in response to water-regime in monoculture

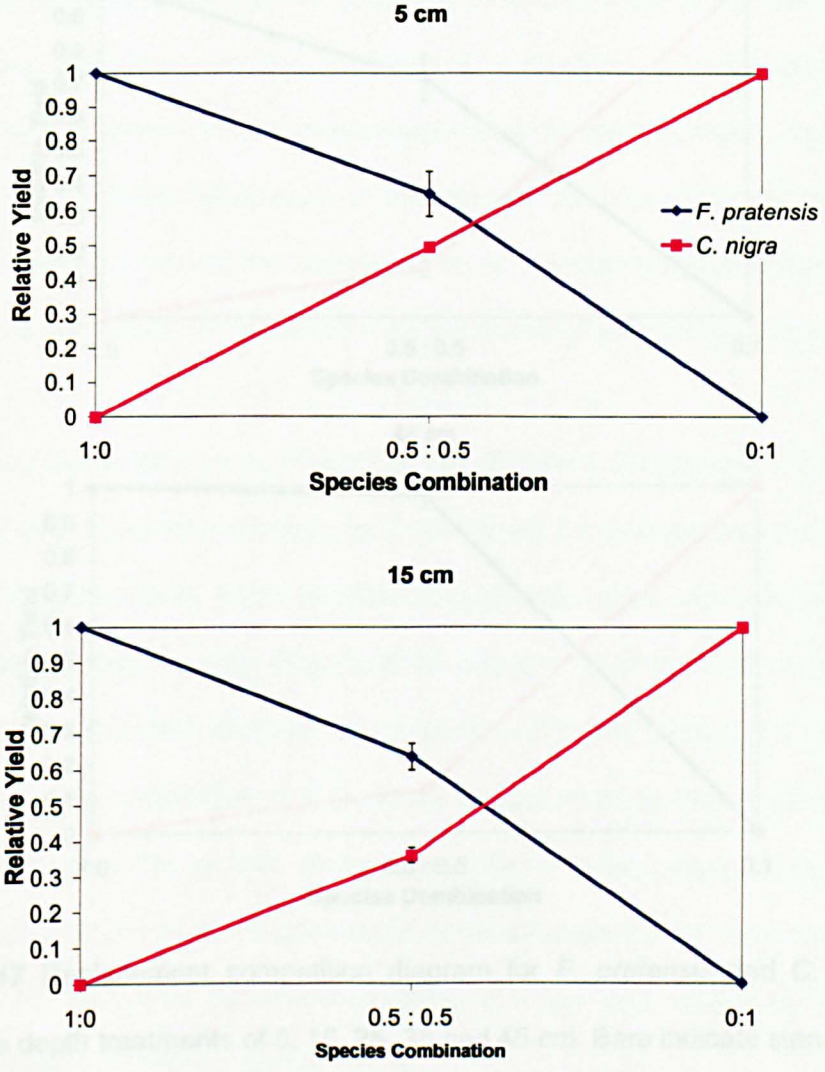
Nitrogen uptake for *C. nigra* and *F. pratensis* in monoculture do not show marked difference between each other in their preferred zone of water-table depth within the range studied. Both species show highest biomass production at a tension of 15 cm (Figure 3.7). This observation shows that the physiological performance of the two species coincides in monoculture. This concurs with earlier studies like that of Ellenberg (1954 cited by Austin, 1990). Ellenberg tested species response along a water-table gradient from 0 to 140 cm and found that the biomass optima of the species tended to coincide in monoculture.

3.4.2 Plant biomass production in response to water-regime in mixture

When plants are grown individually their response to environmental factors doesn't necessarily indicate they will respond similarly when grown with neighbours (Cahill and Casper, 1999). Similarly in this study, the response of both *F. pratensis* and *C. nigra* as individual species in mixture showed a shift from that of monoculture. Skewing is seen with *C. nigra* shifting towards the lower tension, *i.e.* wetter and *F. pratensis* to the higher tensions, *i.e.* drier. This may be illustrated by examining the relative yield (Figure 3.9). Such skewing of realized niche in mixture on clearly defined responses has been mentioned by other investigators (Tilman, 1987; Austin, 1990). Moreover, these shifts in optima corresponded to phytosociological observations of the studied species relative performance in the field (Gowing *et al.*, 2002; also section 4.3.1 in Chapter 4).

In more detail, it is also possible to examine the yield of the two species relative to each other (as shown next in Figure 3.17). As water-table depth increased divergence in the relative yield of the two species is observed.

The replacement competition charts show the grass *F. pratensis* performing better than the sedge *C. nigra* as the soil gets drier. This is to be expected as sedges are known to dominate in wet sites where competition from other species is least (e.g. Grime *et al.*, 1988).



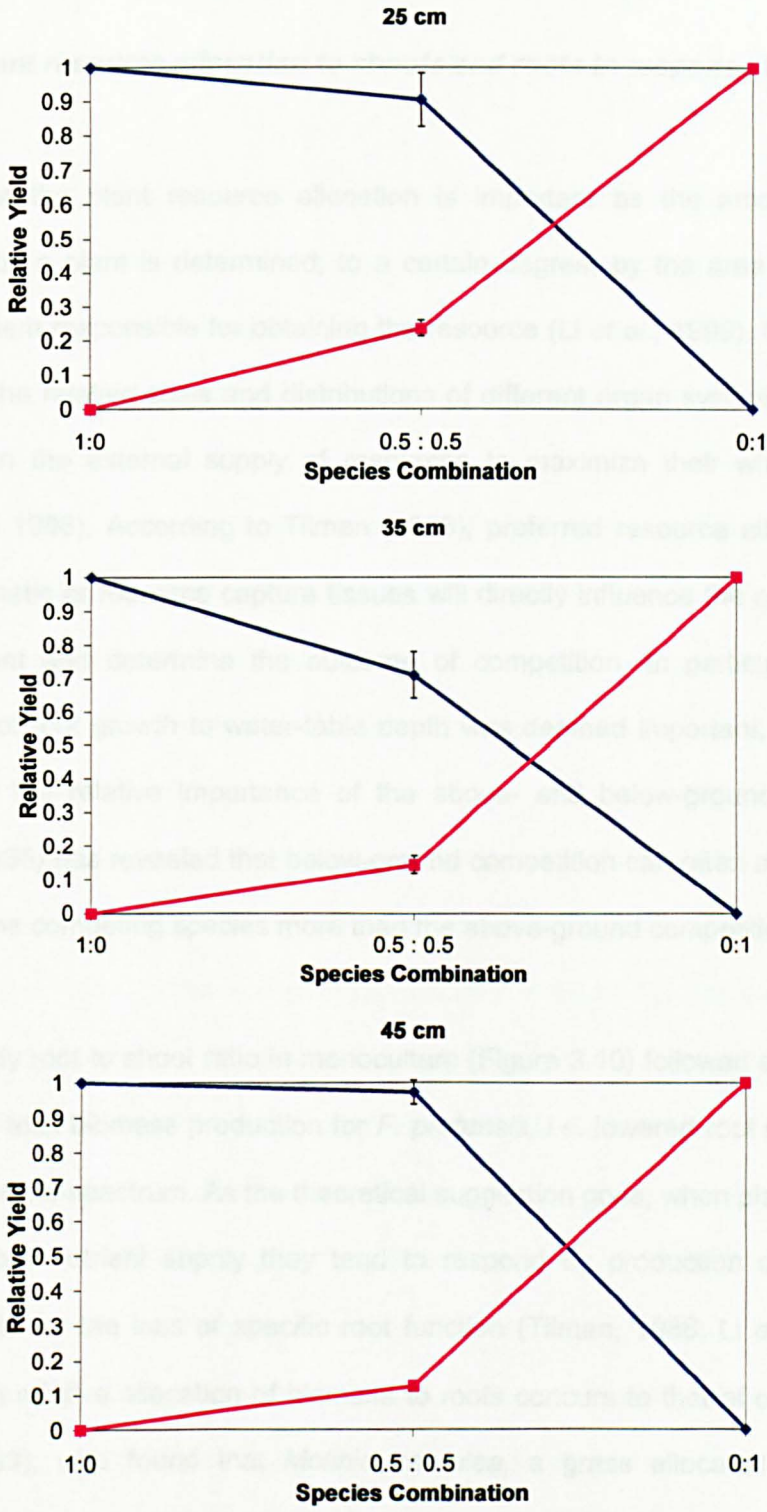


Figure 3.17 Replacement competition diagram for *F. pratensis* and *C. nigra* along water-table depth treatments of 5, 15, 25, 35 and 45 cm. Bars indicate standard error.

3.4.3 Plant resource allocation to shoots and roots in response to water-regime

Looking at the plant resource allocation is important as the amount of resource captured by a plant is determined, to a certain degree, by the area or volume of its organ system responsible for obtaining the resource (Li *et al.*, 1999). Plants are known to adjust the relative sizes and distributions of different organ systems in response to changes in the external supply of resources to maximize their whole-plant growth (Robinson 1986). According to Tilman (1988), preferred resource allocation to either photosynthetic or resource capture tissues will directly influence the competitive ability of the plant and determine the outcome of competition. In particular studying the response of root growth to water-table depth was deemed important, as an extensive review on the relative importance of the above- and below-ground competition by Wilson (1988) has revealed that below-ground competition can often affect the balance between the competing species more than the above-ground competition.

In this study root to shoot ratio in monoculture (Figure 3.10) followed a similar trend as that of the total biomass production for *F. pratensis*, *i.e.* lowered root production at the drier end of the spectrum. As the theoretical suggestion goes, when plants are stressed with reduced nutrient supply they tend to respond by production of more roots to compensate for the loss of specific root function (Tilman, 1988; Li *et al*, 1999). This increase in relative allocation of biomass to roots concurs to that of other authors like Aerts (1999), who found that *Molinia caerulea,* a grass allocated relatively more biomass to the roots at low nutrient supply, thus increasing its competitive ability for below-ground resources. An extensive review of many such studies by Reynolds and D'Antonio (1994) also found similar results.

3.4.4 Plant tissue nutrient concentrations

The plants in this study were sufficiently supplied with other nutrients with only nitrogen limiting. This has been achieved by appropriate preparation of the loam as well as application of modified Long Ashton nutrient solution to ensure no other nutrients including micronutrients are not limiting (see methods section 3.2.4).

The tissue nutrient concentration response showed tissue nitrogen concentration is significantly influenced by soil water-table depth (Figures 3.12a and b). Tissue phosphorus concentration was also found to respond to water-table depths (Figure 3.13a and b). However potassium did not, as can be seen in Figures 3.14a and b.

Liebig's Law of the Minimum states that plant response is determined by that of the least available of the essential nutrients, regardless of the presence of others. Hence in this uptake of major nutrients, a question arises if whether it is nitrogen or phosphorus or both that is limiting the production. The extractable phosphorus of the soil used was 35 ppm, which is more than the 15 ppm considered adequate for plant growth (Landon, 1991). On the other hand, total nitrogen in the soil was 0.11%, less than the 0.2 – 0.5 % considered sufficient for plant growth. This is supported also by the fact that application of external nitrogen increased biomass production (Figure 3.15 and 3.16). Lastly, the tissue nitrogen to phosphorus (N:P) ratio, an index of nitrogen / phosphorus limitation (Verhoeven *et al.*, 1996), was used to identify the limiting nutrient. The N:P ratio for *F. pratensis* and *C. nigra* was 3 and 7 respectively, both are below 16, the cut off ratio that indicates nitrogen limitation. A ratio of > 16 would have indicated phosphorus limitation.

In view of the above evidence, it can be concluded that nitrogen is the nutrient driving the plant response.

3.4.5 Effect of nitrogen fertilization on the influence of water-regime

It is known that the availability of nutrients can have important effects on the competitive relationship between different plant species (e.g. Berendse, 1983; Huckle *et al.*, 2002).

Examination of the influence of water-regime on plant response, individually, under fertilized and fertilized conditions showed significant response as summarized in Table 3.14. The analyses of variance conducted show that the significant influence of water regime on plant biomass production (for *C. nigra*) and tissue nutrient concentrations (for *S. officinalis*) was switched off by fertilization. This switching off suggests that nitrogen is the most likely reason mediating the response to water regime.

On the other hand, the study on the two species, relative to each other, as studied by their relative yield under fertilized and unfertilized conditions is given in Figure 3.18.

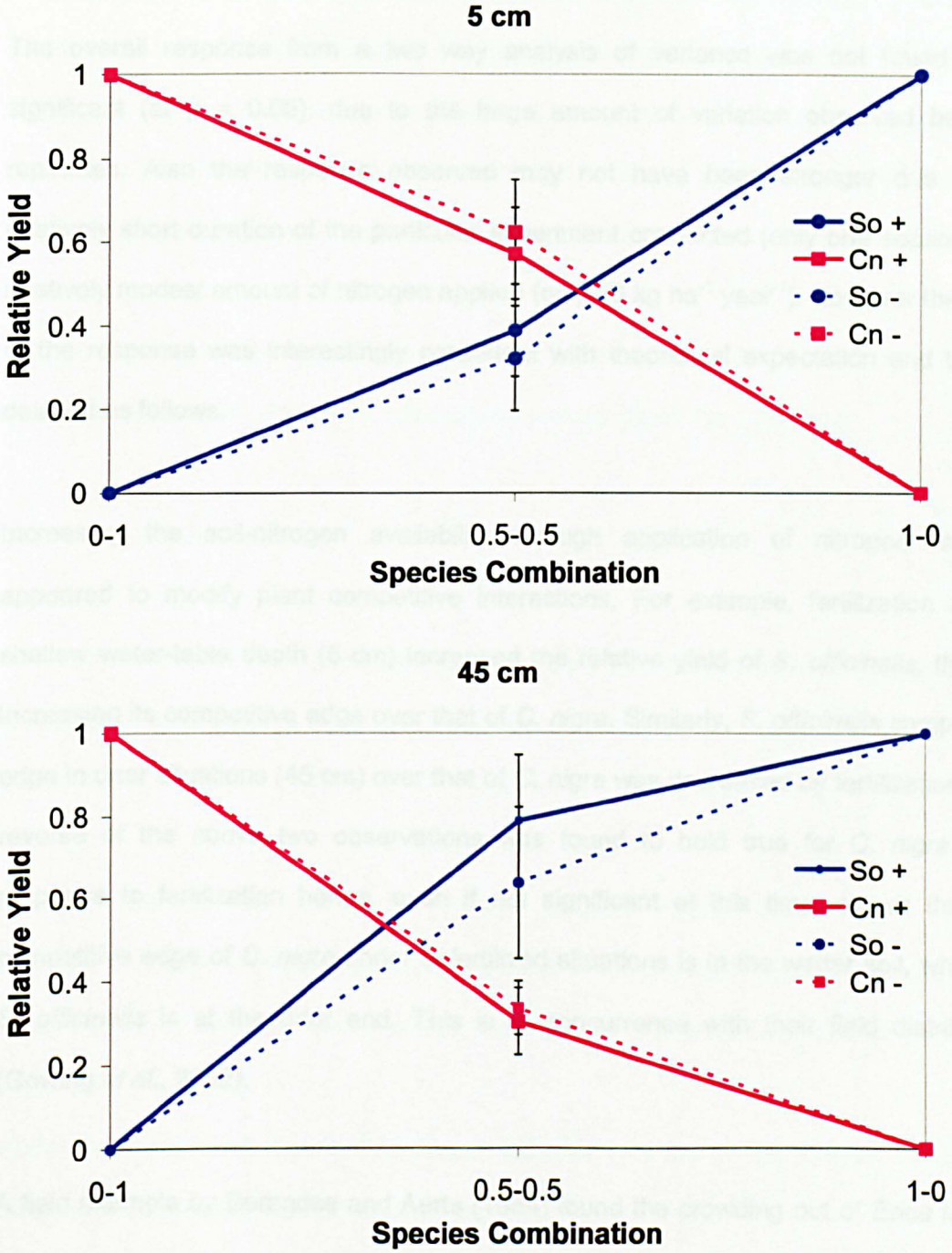


Figure 3.18 Replacement competition diagrams for *S. officinalis* (So) and *C. nigra* (Cn) response with and without fertilization at 5 and 45 cm water-table depth. Solid line and (+) indicate fertilization while broken line and (-) no fertilization.

The overall response from a two way analysis of variance was not found to be significant (at p = 0.05), due to the large amount of variation observed between replicates. Also the response observed may not have been stronger due to the relatively short duration of the particular experiment conducted (only one season) and relatively modest amount of nitrogen applied (only 56 kg ha^{-1} $year^{-1}$). However the trend of the response was interestingly consistent with theoretical expectation and thus is detailed as follows.

Increasing the soil-nitrogen availability through application of nitrogen fertilizer appeared to modify plant competitive interactions. For example, fertilization at the shallow water-table depth (5 cm) increased the relative yield of *S. officinalis*, thereby increasing its competitive edge over that of *C. nigra*. Similarly, *S. officinalis* competitive edge in drier situations (45 cm) over that of *C. nigra* was decreased by fertilization. The reverse of the above two observations was found to hold true for *C. nigra*. This response to fertilization hence, even if not significant at this time, shows that the competitive edge of *C. nigra* under unfertilized situations is in the wetter soil, while for *S. officinalis* is at the drier end. This is in concurrence with their field distribution (Gowing *et al.*, 2002).

A field example by Berendse and Aerts (1984) found the crowding out of *Erica tetralix* by *Molinia caerulea* due to an increase in soil-nitrogen availability. The increase in nutrient availability in this case was induced by both drainage and fertilization. Thus it was found that only on extremely poor, water-logged sites, where nitrogen availability was reduced could *E. tetralix* survive as the competitive ability of *M. caerulea* was greatly reduced. Similarly, Neill (1990) studied whitetop grass *Scolochloa festucacea*

and cattail *Typha glauca* response to fertilization at shallow and deep water levels in a prairie marsh. A reversal of species biomass production under shallow and deep water levels was obtained with fertilization. Similar to the study by Berendse and Aerts (1984) a study on salt marsh species *Spartina* and *Puccinellia* by Huckle *et al.* (2002) showed coexistence was possible at reduced nutrient availability or increased immersion.

Overall these findings tend to support the Goldberg (1990) competition model *i.e.* competition for direct resources, nitrogen apparently being the case here.

3.5 Conclusion

Competition for nutrients has often been shown to be responsible for dictating plant interactions and consequently the regulation of species composition in grassland communities.

In this study, it was shown that plants respond to soil water-regime in both aboveground and belowground biomass production. The relative response in yield was also found to be dependent on whether plants were grown in monoculture or mixture.

Within the plant itself, resource allocation between root and shoot tissues varied as a response to minor differences in water-regime.

The application of external nitrogen through fertilization negated the influence of water-regime on plant response. However there were only indications of negation on plant competitive interaction.

Chapter Four

4 Influence of water-regime and soil-nitrogen availability on the composition of species-rich meadow community

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In this chapter, a species-rich wet meadow, Cricklade North Meadow National Nature reserve is monitored for its species distribution along gradients of water-regime and soil-nitrogen availability.

4.1 Introduction

Plant communities in wet meadows are substantially influenced by the depth and annual variation of the water-table (Hayati and Proctor, 1990; Gowing and Youngs, 1997, Dwire *et al.*, 2004). Recent experimental and observational field studies have shown that minor shifts in water-regime may play a significant role in the composition of plant communities (e.g. Gowing and Spoor, 1998; van Oorschot *et al.*, 2000). It is also known that, in many natural environments, nutrient availability is the major factor affecting species composition and the dynamics of plant communities through its effect on competitive interactions (e.g. Berendse, 1983; Aerts, 1999). Earlier studies in the relationship of water-regime and nutrient availability have mostly focused in manipulative experiments whereby changes are introduced via drainage (e.g. Grootjans *et al.*, 1986; Olde Venterink *et al.*, 2002a), raising water-table (e.g. Oomes *et al.*, 1996) or application of fertilizer (e.g. Joyce, 2001, Rajaniemi, 2002). Alongside this the influence of water-regime on soil chemical properties e.g. nutrient availability in meadow systems have been investigated (Neill, 1990; Castelli *et al.*, 2000, van Oorschot *et al.*, 2000). Specifically this study focuses on a meadow that has not been directly manipulated. The main soil nutrient selected for this study was nitrogen, as its availability is highly influenced by water-regime (see Chapter 2).

The aim of this chapter is to understand the relationships between water-regime and soil-nitrogen availability on plant community response. In this context, the influence of water-regime and nitrogen availability was studied by monitoring plant distribution, biomass production and soil-nitrogen availability along an existing gradient of fine-scale heterogeneity in soil water-regime.

4.2 Methodology

4.2.1 Field site

Cricklade North Meadow National Nature Reserve in Wiltshire (National grid reference SU096958) lies on the alluvial deposits of the floodplains of the River Thames and the River Churn. The meadow covers an area of 44.4 ha and is owned by English Nature. The reserve is internationally important for its many different plants, including the largest British population of snake's-head fritillary (*Fritillaria meleagris*). It has thus been designated as a Site of Special Scientific Interest (SSSI) and candidate Special Area of Conservation (cSAC). For hundreds of years, Cricklade NNR has been traditionally managed as a hay meadow. This management consists of a hay cut in midsummer followed by cattle grazing as a common land after August 12th. The animals are then removed before the 12th February of the following year so that the vegetation can start its growth cycle.

The status of the site and level of protection granted to it called for employing careful non-disruptive study methods. In addition to designing appropriate methods, data from a long running DEFRA-commissioned project by Gowing *et al.* (2002) were utilised, and are acknowledged where they occur.

The DEFRA study involving hydrology and botanical survey encompassed 120 (1 m × 1m) quadrats covering a block of approximately 250 m by 250 m. For the nutrient monitoring and plant tissue analysis in this investigation, 44 quadrats along 4 transects were selected. The location of the quadrats in the field site is given in Figure 2.1.

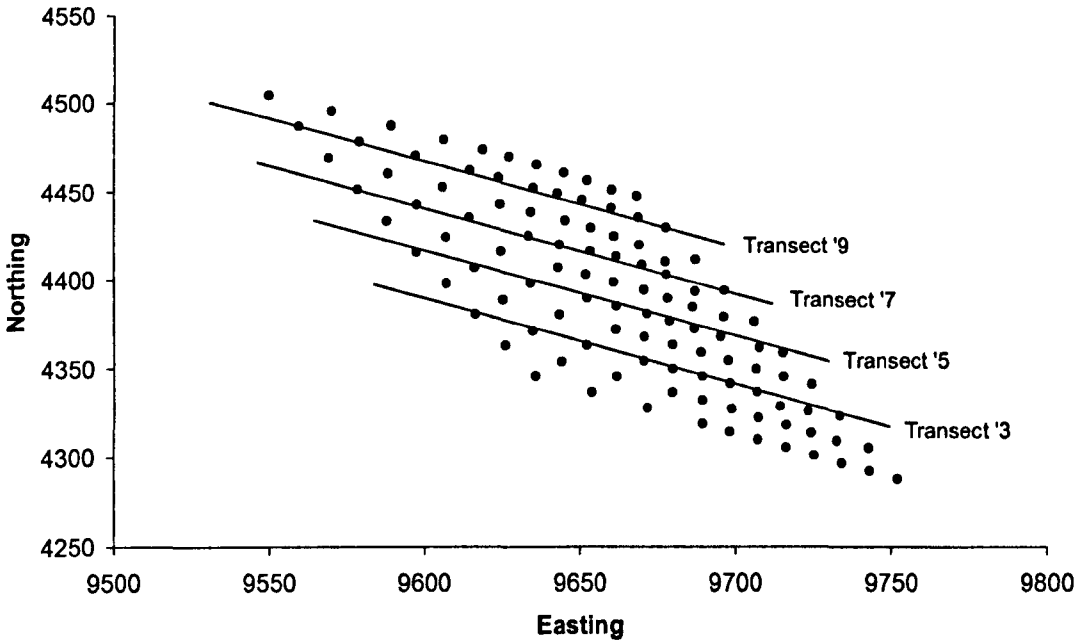

Figure 4.1 Grid reference and layout of quadrats at Cricklade NNR field site. Four transects were studied. (•) denotes quadrat location.

4.2.2 Monitoring soil water-regime

Accurate understanding and monitoring of the water-table behaviour within the field and specifically at the position of each quadrat was necessary to correlate soil nutrient status, botanical distribution and water-regime.

Water-table positions were estimated using a site-specific hydrologic model. The model used was based on a 'shallow aquifer water-table model' developed for alluvial soils overlying permeable sand and gravel deposits with hydraulic connection to surrounding rivers (Gowing *et al.*, 1998; Gowing *et al.*, 2002). The model also incorporates modifications for resistance to flow due to soil compaction near the river banks and siltation on the river bed. The output from the model was in the form of weekly

estimates of water-table elevation at each quadrat location. These data were then summarized and interpreted through the concept of Sum Exceedence Values (SEV) for aeration and water stress (Sieben, 1965 cited in Gowing and Spoor, 1998).

The SEV method relies on two threshold depths uniquely calculated for any particular site. The first threshold defines the water-table depth at which the zone of densest rooting (taken to be 0-100 mm depth) begins to become water-logged, and the second defines when drying of the surface soil becomes detectable by plants. The water-logging threshold is calculated from the soil moisture release curve as the depth that gives 10% air-filled porosity. The soil drying threshold is calculated using Richard's equation (Gardner, 1958 cited by Gowing and Spoor, 1998) as the depth that gives 50 cm (5 kPa) tension at the soil surface, *i.e.* where plants start to show effects of water stress (Henson *et al.*, 1989). For Cricklade NNR soils, the thresholds for the above stresses were set as 40 cm for aeration stress and 45 cm for drying stress (Gowing and Spoor, 1998). For each threshold, the SEV represents the degree to which water-tables exceed it. SEV is only cumulated during the period of active plant growth (March – September inclusive), when the plants are most sensitive to the stresses. Hence, the SEV_a, for aeration stress *i.e.* water logging and the SEV_d for soil drying are obtained.

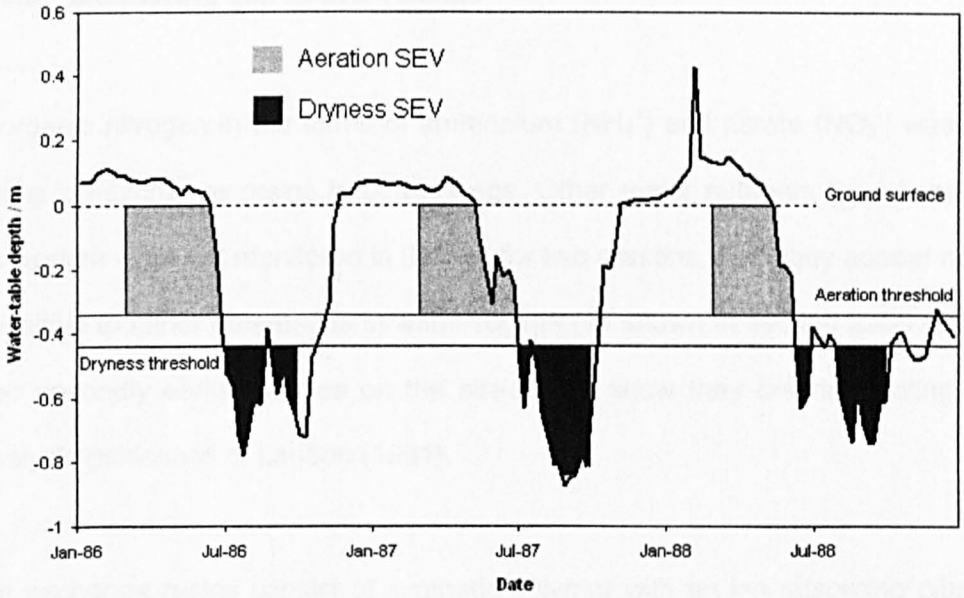

Figure 4.2 A sample Sum Exceedence Value (SEV) calculation diagram

The advantage of using the SEV approach is that it accounts for and cumulates the extent as well as duration of stress. Moreover, use of site-specific thresholds means that the resultant information is transferable between different sites. Therefore it enables to compile preferred zones of species occurrence from a large number of different botanical surveys (Gowing *et al.*, 2002).

For monitoring nitrogen availability, mean water-table depths were extracted from the weekly model output. This was preferred as the duration of the nutrient monitoring was relatively short, i.e. 6 weeks, and hence the SEV values of the whole growing season wouldn't have been appropriate.

4.2.3 Monitoring soil nutrient status

Inorganic nitrogen in the forms of ammonium (NH_4^+) and nitrate (NO_3^-) was monitored using ion-exchange resins in buried bags. Other major nutrients like phosphorus and potassium were not monitored in the soil for two reasons. First they appear not to be so sensitive to minor differences in water-regime (as shown in section 2.4.5 of Chapter 2) and secondly earlier studies on the site's soils show they are not limiting using soil analysis guidelines of Landon (1991).

Ion exchange resins consist of synthetic polymer with an ion adsorbing capacity. The principle behind the resin is a simple reversible ion exchange reaction, with ions in the environment being taken up until equilibrium is achieved. Before the start of the experiment counter-ions with low affinity to the resin are used to saturate the resin and later on a strong elution solution is used to displace the ions taken up during the experiment (Skogley and Dobermann, 1996; Qian and Schoenau, 2002).

Ion exchange resins have been used for *in situ* nutrient availability studies under relatively undisturbed conditions in both field and laboratory conditions (e.g. Binkley and Matson, 1983; Davidson *et al.*, 1990; Sherrod *et al.*, 2003). They are generally installed in the soil horizon for a certain time period, then recovered and extracted, to inform about nutrient availability during that period. Measurements of available soil-nitrogen with resin bags are also considered to be more relevant to plants than other methods because resin measurements integrate over time, are sensitive to the different mobilities of nitrate and ammonium ions in the soil, and are sensitive to soil moisture (Giblin *et al.* 1994). Ion exchange resin measurements of nitrogen have also been

shown to correlate with standard soil-nitrogen analysis methods (e.g. Binkley and Matson, 1983).

In this study, loose ion exchange resin beads (1 mm diameter, Acros®organics BVBA, Geel, Belgium) were sewn in 6 × 5 cm nylon mesh bags. A cation exchange resin Amberlite® IR-120, initially saturated with sodium counter-ions and an anion exchange resin Amberlite® IR-900, initially saturated with chlorine counter-ions were used. A 10 g fresh weight of IR-900 (6.3 g dry weight) and 10 g of IR-120 (4.8 g dry weight) in separate bags were used for each data point. This quantity of resins was sufficient to accumulate the expected quantity of ions in the field. This was calculated from the exchange capacity of the resin and ascertained by pilot experiments in the field. The resin bag data points were collected from a minimum of three and a maximum of four sides of the quadrats used for botanical studies. For installation a slit was made in the ground using a trowel 5 - 8 cm below the soil surface and the bags inserted. The points were marked with a 5 cm long galvanized iron pipe and a plant label to enable detection with a metal detector (see Figure 4.3). A total of 150 bags were placed over 4 transects in 44 quadrats. The resin bags were then left in the ground between April 28 and June 9th 2003 for a duration of 42 days. This particular time was selected because it lies within the active plant growth season as well as the time considered most representative for measuring nitrogen availability (Schaffers, 2000).

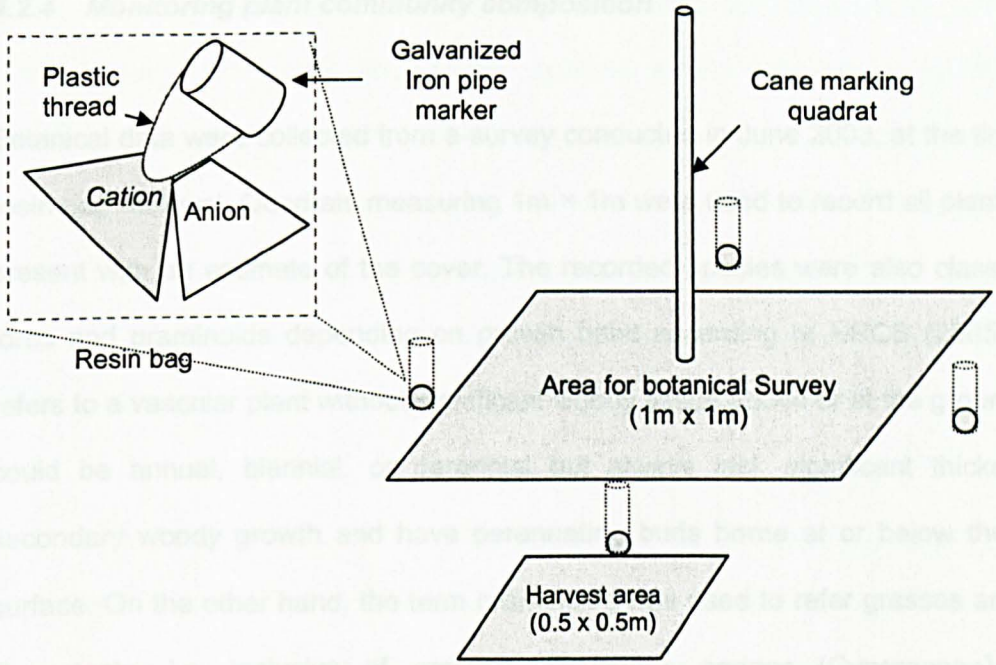

Figure 4.3 Schematic diagram of resin bags and placement within each study quadrat

Extraction involved emptying the resin bags and shaking the resin with 50 ml of aqueous 2 M KCl solution at 110 rpm for an hour (Binkley *et al.*, 1992). The extract was then filtered using Whatman® No.42 filter paper and stored at -20 °C until analysis. Analysis was done using high capacity exchange columns; namely IonPac CS16 (Dionex®, Sunnyvale, California) a high-capacity cation exchange column for ammonium nitrogen, and an anion-exchange column IonPac AS9-HC for nitrate-nitrogen, in a Dionex DX-100 (Dionex®, Sunnyvale, California) Ion Chromatograph machine. Before statistical analysis, the data on soil-nitrogen availability were transformed using the $\log_{10}$ (x+1) function to make them normally distributed. Median nitrogen availability per quadrat was used when relating soil-nitrogen availability with quadrat data. The median was preferred over the mean to account for the variability while reducing the effect of very high and very low (zero) values recorded.

4.2.4 Monitoring plant community composition

Botanical data were collected from a survey conducted in June 2003, at the time of the resin bag retrieval. Quadrats measuring 1m × 1m were used to record all plant species present with an estimate of the cover. The recorded species were also classified into forbs and graminoids depending on growth habit according to NRCS (2005). A forb refers to a vascular plant without significant woody tissue above or at the ground. Forbs could be annual, biennial, or perennial but always lack significant thickening by secondary woody growth and have perennating buds borne at or below the ground surface. On the other hand, the term graminoids was used to refer grasses and grass-like plants, i.e. inclusive of grasses (Poaceae), sedges (Cyperaceae), rushes (Juncaceae).

For dry matter production, harvest was made at 2 cm aboveground over an area of 50 cm by 50 cm, adjacent to the quadrat used for botanical survey. This harvest coincides with the first traditional hay-cut. The harvested plant matter was then taken to the laboratory for determination of moisture content, weight of dry matter and nutrient concentration. This was done by drying a sub-sample at 55 °C for 72 hours. Tissue nutrient analyses for carbon, nitrogen, phosphorus and potassium were then made after grinding the dried matter.

The analysis for carbon and nitrogen was done with LECO-2000® Elemental Analyser. A 0.2 g sample of plant matter was mixed with an accelerator flux and combusted. The concentration of carbon and nitrogen was then determined from the gases evolved. For tissue phosphorus analysis, 0.5 g plant material was dry ashed in a muffle furnace at 450 °C for five hours. The ash was then mixed with 5 ml of 2N hydrochloric acid and

made up to 50 ml with deionised water in a volumetric flask (Ryan *et al.*, 2001). After filtering through Whatman® No. 42 filter paper the extract was then mixed with Barton yellow colour complex (MAFF, 1986) and the resulting colour determined using Helios Thermo Spectroscopic colorimeter at 410 nm wavelength. Plant tissue potassium content was analysed using flame photometry (Gallenkamp® SGA_330C Loughborough, UK), after dry ashing 0.5g of plant material and dissolving with 5ml of 2N hydrochloric acid and deionized water on 50 ml volume.

Community response to water-regime and soil-nitrogen availability gradient was investigated through mean weighted Ellenberg scores (Ellenberg, 1979). Ellenberg scores are a series of subjective ecological scores on a scale of 1-9 (or in some cases 12) frequently employed to reflect tolerance of plants to selected environmental conditions. In this case, the Ellenberg scores for moisture (F) and soil fertility or nitrogen (N) were used as calculated for British flora by Hill *et al.* (1999).

Lastly, multivariate ordination analysis using Canonical Correspondence Analysis (CCA) technique was performed on the distribution of all species recorded in the botanical survey. The input variables for this were harvest data of dry matter production and tissue nutrient concentrations (nitrogen, phosphorus and potassium) along with environmental gradients of water-table depth and soil-nitrogen availability.

4.3 Results

4.3.1 Water-regime and plant species distribution

The distribution of *C. nigra*, *F. pratensis* and *S. officinalis* along the prevailing water-regime gradient is shown in Figure 4.4 and 4.5.

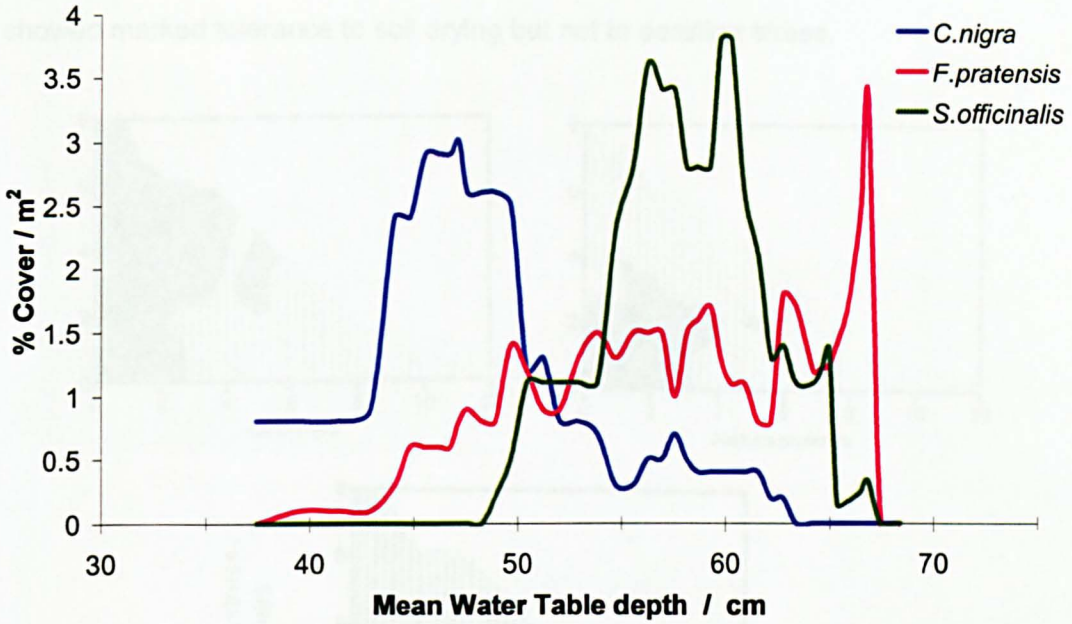

Figure 4.4 Distribution of *F. pratensis*, *C. nigra* and *S. officinalis* along mean water-table depth, between April 28th- June 9th, 2003 as rolling average (n=44)

The % cover of *F. pratensis, C. nigra* and *S. officinalis* from season 2003 within the monitored range of 35 – 70 cm mean water-table depth showed distinct regions favouring each species. *Carex nigra* dominated at the relatively wetter end (<50 cm), *F. pratensis* on the drier end (> 63 cm) while *S. officinalis* occupied the middle range (55 - 63 cm). All three species appear to coexist equally at 50 - 55 cm water-table depth.

Similarly long term monitoring of the water-regime expressed as sum-exceedence values (Gowing *et al.*, 2002) for drying and aeration stress shows the preferred niches of the three species (Figure 4.5). The charts were created by determining where a species occurs significantly more often than by chance ($p<0.05$) from a large set of data. *Carex nigra* occupied a region of tolerance to aeration stress, but is found not to compete well where there is substantial soil drying. *Festuca pratensis* occupied a

region of little tolerance to either soil drying or soil aeration stress, while *S. officinalis* showed marked tolerance to soil drying but not to aeration stress.

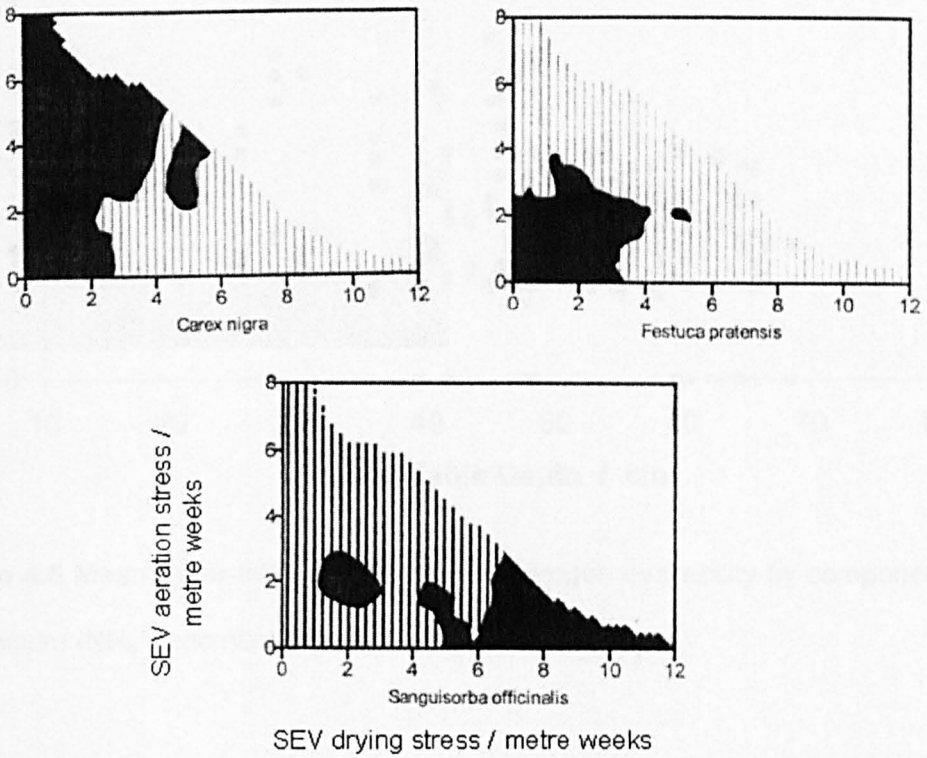

Figure 4.5 Preferred ranges of the common sedge *Carex nigra*, meadow fescue *Festuca pratensis*, and greater burnet *Sanguisorba officinalis* along SEV axes for soil drying stress and aeration stress (after Gowing *et al.*, 2002).

4.3.2 Water-regime and soil-nitrogen availability

Relationship of available soil-nitrogen with mean water-table depth is shown in Figure 4.6.

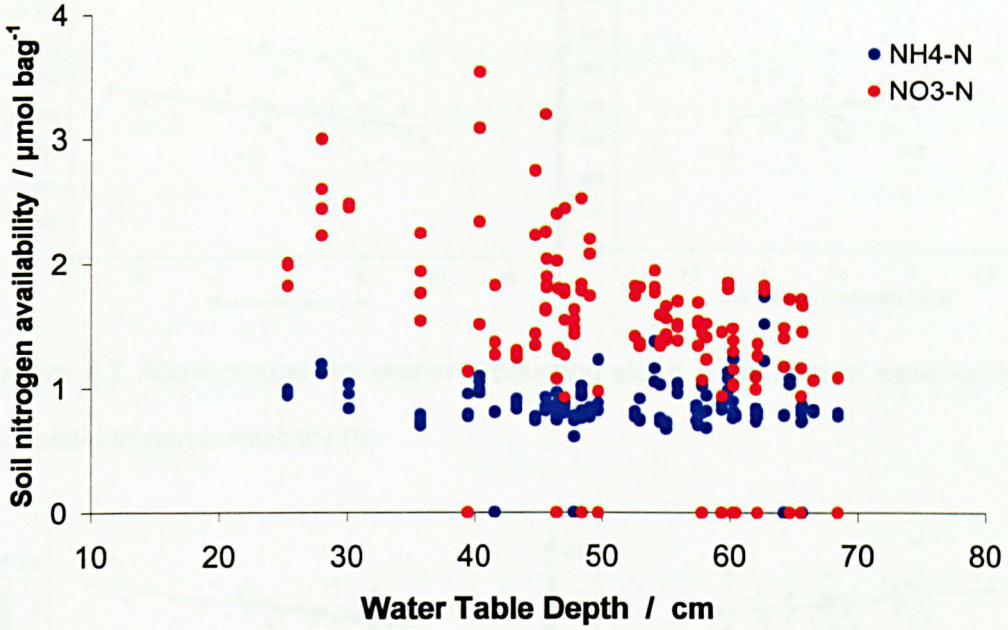

Figure 4.6 Mean water-table depths against nitrogen availability by component forms: ammonium (NH_4^+) and nitrate (NO_3^-).

Soil-nitrogen availability, in relation to mean water-table depth showed a significant negative relationship ($r = -0.53$, $p < 0.001$). Maximum soil-nitrogen availability within the observed range of 20 - 80 cm mean water-table depths appears to be at about 40 cm. Nitrogen availability is seen to decline when water-table depth increases more than 50 cm and slightly when it decreases below 40 cm.

4.3.3 Water-regime and soil-nitrogen availability on dry matter production and tissue nutrient concentrations

Plant response in the form of dry matter production and tissue nutrient concentrations to water-table depth and soil-nitrogen availability is given in Table 4.1. Linear regression was also used to describe the significant relationships (Figures 4.7-4.9).

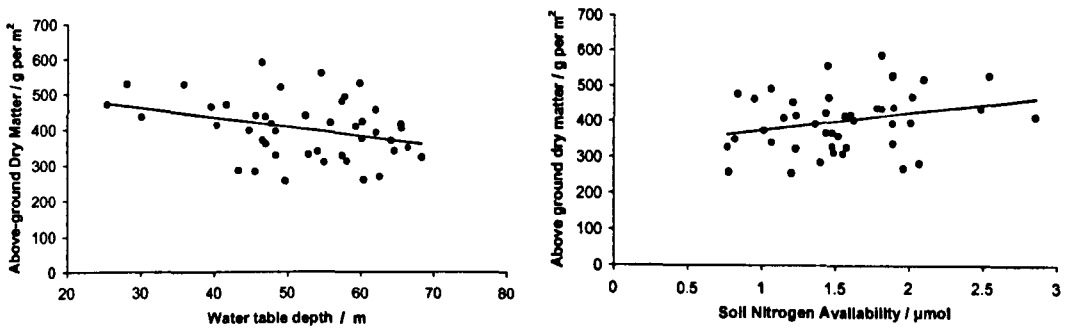

Figure 4.7 Aboveground dry matter production along a gradient of water-table depth (a), soil-nitrogen availability (b)

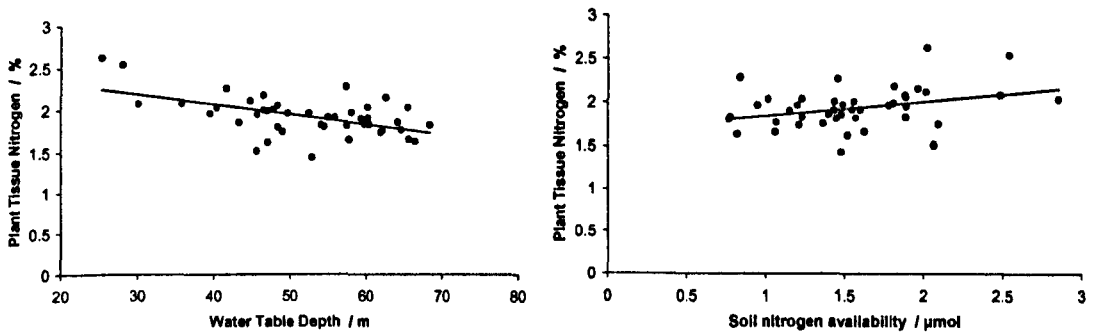

Figure 4.8 Plant tissue nitrogen concentration along a gradient of water-table depth (a), soil-nitrogen availability (b)

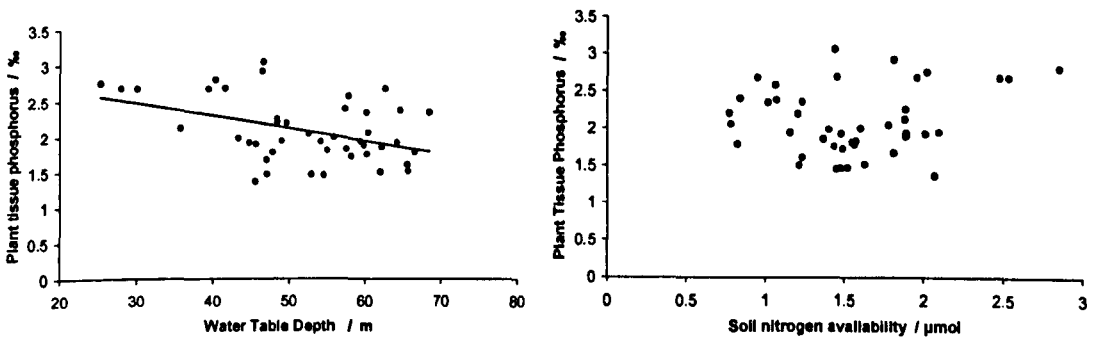

Figure 4.9 Plant phosphorus tissue concentration along a gradient of water-table depth (a), soil-nitrogen availability (b)

Table 4.1 Comparison of variability explained by water-regime and soil-nitrogen availability (n = 44). Correlation coefficient and probabilities (p) are given as r and (p)

Item	Water-regime	Soil-nitrogen availability
Above-ground dry matter	-0.33 (0.03)	0.28 (0.07)
Tissue nitrogen (%)	-0. 54 (<0.001)	0.31 (0.04)
Tissue Phosphorus (‰)	-0.63 (<0.001)	0.13 (0.41)
Tissue Potassium (‰)	-0.08 (0.59)	0.09 (0.55)
N:P ratio	0.15 (0.35)	0.05 (0.76)
C:N ratio	0.51 (<0.001)	-0.31 (0.04)

As can be seen from Table 4.1, water-regime significantly influenced dry matter production, tissue nitrogen and phosphorus concentration, as well as tissue carbon to nitrogen ratio. On the other hand, soil-nitrogen availability significantly influenced dry matter production, nitrogen concentration, carbon to nitrogen ratio. Step-wise regression showed water-regime gave the best explanation for all those measured.

4.3.4 Water-regime and nitrogen availability on community response

A total of 61 species were recorded in the 44 quadrats studied. Water-table depth was significantly correlated to species-richness ($r=0.51$ $p<0.01$), with a large number of species associated with drier soil (See Figure 4.10a). The relationship between plant species-richness and soil-nitrogen availability was also significant ($r=-0.35$ $p=0.02$) with most species associated with lower available nitrogen (See Figure 4.10b).

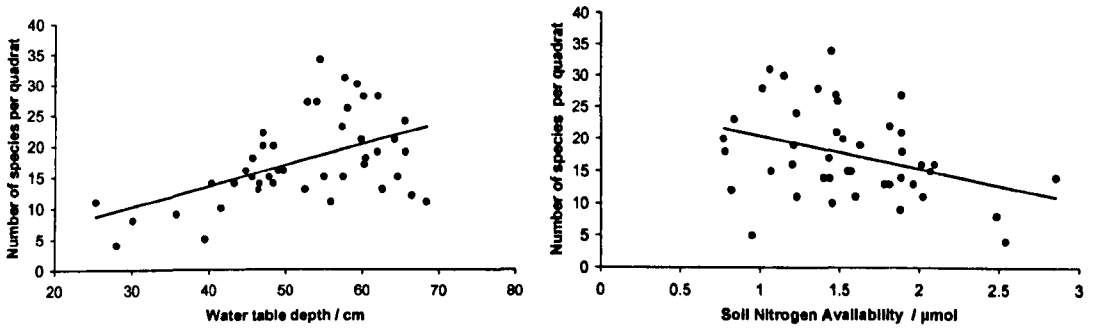

Figure 4.10 Plant species-richness along a gradient of water-table depth (a) soil-nitrogen availability (b)

Weighted mean Ellenberg moisture (F) score values from the species encountered during the survey are shown in Figure 4.11a. The mean Ellenberg score for moisture showed negative correlation with mean soil water-table depth ($r=-0.66$, $p<0.01$) and median soil-nitrogen availability ($r = 0.50$ $p < 0.01$).

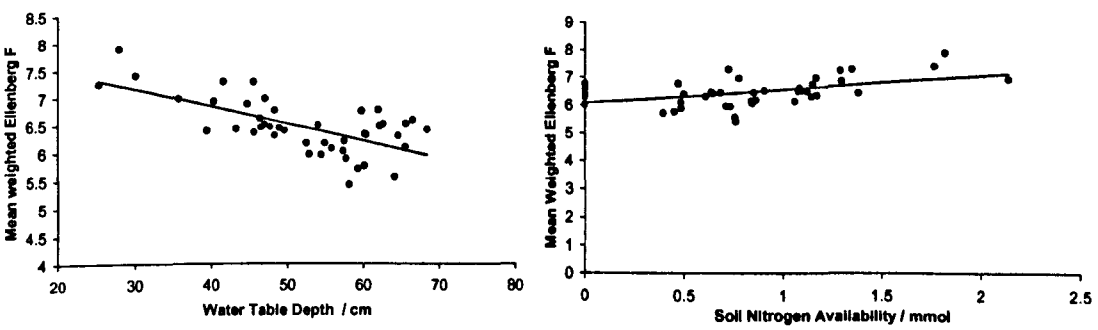

Figure 4.11 Mean weighted Ellenberg moisture (F) score and water-table depth (a), and soil-nitrogen availability (b)

However, Ellenberg scores for nitrogen (N) did not show any relationship with either soil water-table depth or soil-nitrogen availability. See Figures 4.12a and b.

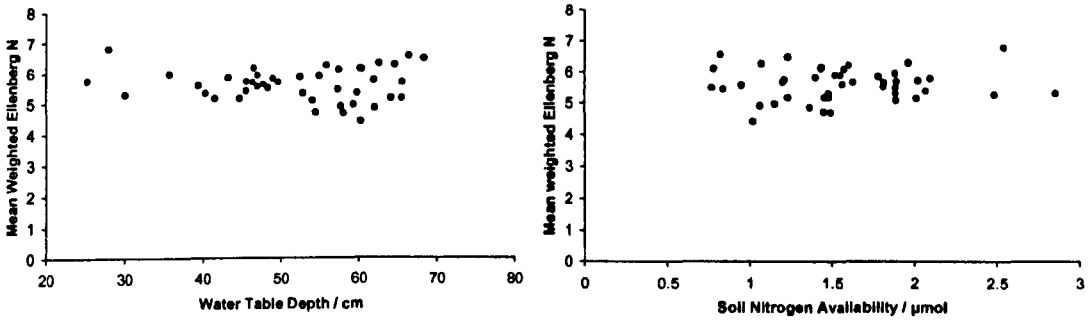

Figure 4.12 mean weighted Ellenberg nutrient status (N) score and water-table depth (a), and soil-nitrogen availability (b)

The distribution of graminoids versus forb species along water-table depth and soil-nitrogen availability gradient is shown in Figure 4.13a and b.

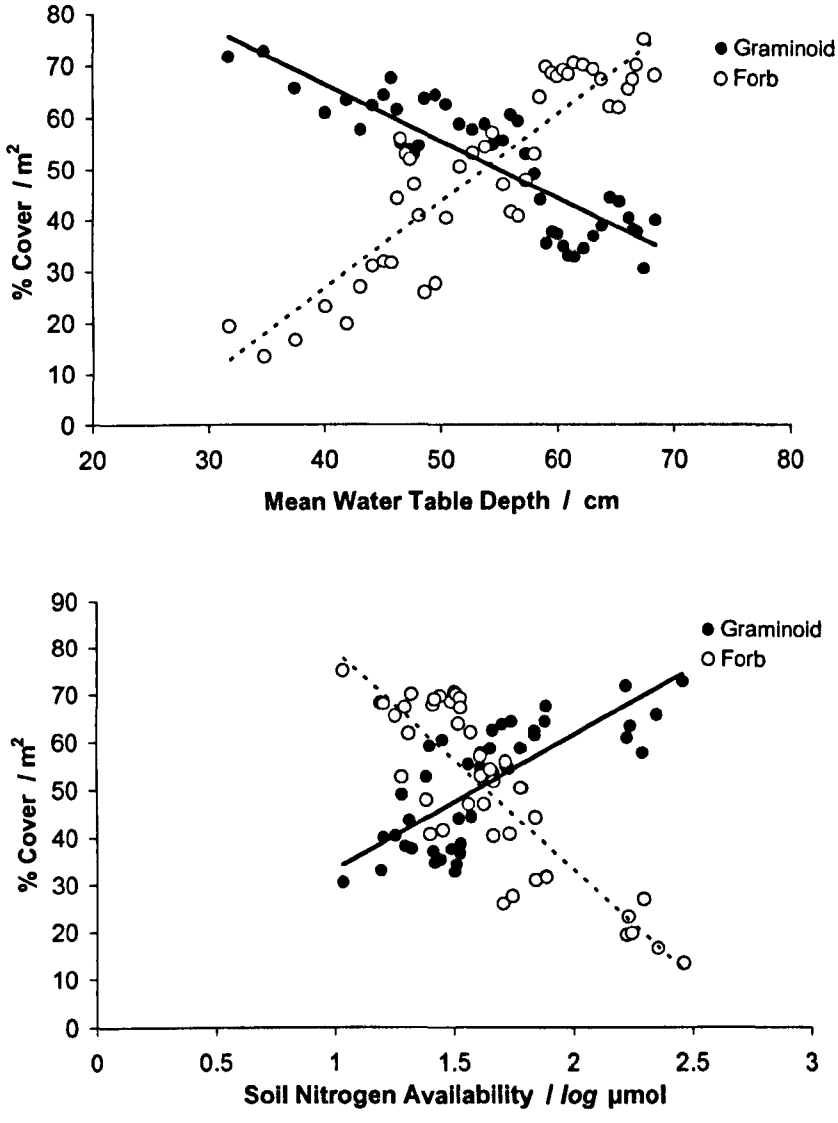

Figure 4.13 Distribution of graminoids and forbs along a gradient of soil water-regime (a) and soil-nitrogen availability (b)

The distribution shows an opposite trend between graminoids and forbs. Graminoids are negatively correlated with water-table depth ($r=-0.86$, $p < 0.001$) and positively with soil-nitrogen availability ($r=0.77$, $p < 0.001$). On the other hand forbs are positively correlated with soil water-table depth ($r=0.89$, $p<0.001$) and negatively with soil-

nitrogen availability ($r=-0.85$, $p<0.001$). The switch in dominance between the two groups of plants occurs at more than 55 cm water-table depth, which coincides with nitrogen availabilities of more than 40 μmol bag^{-1}.

4.3.5 Canonical Correspondence Analysis

Multivariate ordination using canonical correspondence analysis (Figure 4.14) shows the distribution of the species as well as plant aboveground characteristics of dry matter production and tissue nutrient concentration along environmental variables of soil water regime and soil-nitrogen availability. The distribution revealed how species relate to each other in terms of their environmental requirements.

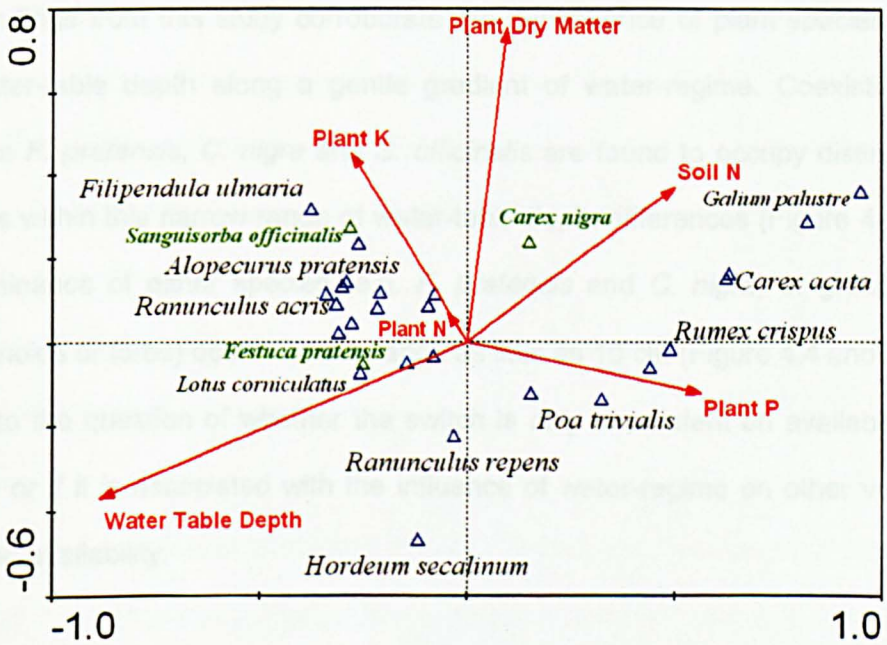

Figure 4.14 Canonical Correspondence Analysis of measured field data (water-table depth and soil-nitrogen availability with plant aboveground dry matter, tissue nutrients) and some common meadow species (only selected few are labelled fully).

4.4 Discussion

Hydrologic conditions determine species composition, distribution, successional trends and primary productivity in wet meadows (Brinson *et al.*, 1981; Howard-Williams, 1985). One mechanism of how hydrology controls the plant community is by influencing the availability of nutrients (Neill, 1990). This could be either directly through its effect by bringing water of differing hydrochemical qualities (Willby *et al.*, 1998), deposition of sediments (Barko and Smart, 1979) or indirectly by influencing mineralization from soil organic matter (e.g. Oomes *et al.*, 1996, 1997).

4.4.1 Water-regime and plant species distribution

The findings from this study corroborate the dependence of plant species distribution on water-table depth along a gentle gradient of water-regime. Coexisting meadow species *F. pratensis*, *C. nigra* and *S. officinalis* are found to occupy distinct preferred regions within this narrow range of water-table depth differences (Figure 4.4). A switch in dominance of either species (e.g. *F. pratensis* and *C. nigra*) or groups of plants (graminoids or forbs) occurs over a range as little as 10 cm (Figure 4.4 and 4.13a). This leads to the question of whether the switch is only dependent on availability of water *per se* or if it is associated with the influence of water-regime on other variables e.g. nitrogen availability.

4.4.2 Water-regime and soil-nitrogen availability

Dependence of soil-nitrogen mineralization on soil moisture regime is a known phenomenon (e.g. Stanford and Epstein, 1974; Myers *et al.*, 1982; Paul *et al.*, 2003).

The sensitivity of nitrogen mineralization to minor differences in water-regime was shown in Chapter 2. The monitoring of field-nitrogen availability, under a range of plant communities repeated here was conducted in early spring along a gradient of water-table depth. The spring season was selected as it heralds the warming of the weather and beginning of plant growth (Broad and Hough, 1993) which facilitates active mineralization (Wong and Nortcliff, 1995). This early phase of resource availability will thus result in intense competition between plants.

The monitoring confirmed the dependence of soil-nitrogen availability on mean water-table depth. Within the range of 20 - 80 cm water-table depth studied the maximum available soil-nitrogen was found between the ranges of 40 - 45 cm. This optimum range for soil mineralization coincides with the stress-free range calculated for this site on the basis of soil physics (see section 4.2.2). Although there were no quadrats with water-table depth of < 20 cm at the monitoring time, a decline in available nitrogen availability would have been expected with the development of severe aeration stress (cf. aeration threshold in section 4.2.2 and section 2.4.3 chapter 2).

4.4.3 Soil-nitrogen availability, aboveground plant dry matter production and tissue nutrient concentration

Aboveground dry matter production and tissue nitrogen concentration increased as a response to increase in soil-nitrogen availability (Figure 4.7 and 4.8). This corresponds to results from nitrogen fertilization and drainage studies, in nitrogen limited sites, where plants responded with increase in dry matter production (Vermeer and Berendse, 1983; Oomes *et al.*, 1996; Olde Venterink *et al.*, 2001; Voisin *et al.*, 2002).

Tissue phosphorus concentration strongly correlated with soil water-table depth but not with soil-nitrogen availability. This may raise the question as to which of the two nutrients is driving the plant response. Firstly, even though phosphorus concentration shows a similar response to nitrogen along the gradient of water-regime in the field, laboratory investigations have shown that it is nitrogen that responds more strongly to small differences in water-regime at these fine-scale differences in water-regime (see section 2.4.1; 2.4.5 Chapter 2). Moreover the response of phosphorus in this meadow is most likely to be dominated by the gradient of flooding by river water, which deposits phosphorus-rich sediment (Gilbert, 2000) rather than mineralization. Secondly, examination of the nitrogen to phosphorus ratio in harvested plant tissue, a parameter often used to study nutrient limitation in ecosystems (Koerselman and Meulman, 1996), further strengthens the role of nitrogen as a limiting nutrient. The N:P ratio from in this site averaged 10 i.e. less than 14, the critical upper limit cited for nitrogen limitation and being no more than 16, the critical lower limit for phosphorus limitation (Verhoeven *et al.*, 1996). On the other hand, potassium uptake in plant tissue was not related to water-table depth or soil-nitrogen availability (see Table 4.1).

4.4.4 Water-regime and nitrogen availability relationships on community response

Established plant communities are a reflection of the environmental conditions they grow in (Dodd *et al.*, 2002). Several indicators are used to describe this aggregate response by plants, such as direct methods like species-richness, diversity, community composition and indirect methods like that of Ellenberg scores (Ellenberg, 1979).

Species-richness increased as the soil water-table depth increased. Higher numbers of species per quadrat were recorded between the water-table depths of 50 to 60 cm with a maximum of 34 species m^{-2}. Wetter soil conditions beyond this region were associated with a reduction in species number (Figure 4.10a). One reason for this could be an increase in the availability of nitrogen as noted above. Higher numbers of species coincided with quadrats having soil-nitrogen availability of less than 40 µmol (Figure 4.10b). The negative relationship of species-richness to increased levels of nitrogen in soil is a well known phenomenon from fertilization and drainage studies (e.g. Vermeer and Berendse, 1983; Mountford *et al.*, 1993; Joyce, 2001; Rajaniemi, 2002) but has not previously been demonstrated in an unfertilized soil varying only in mineralized nitrogen availability. The reduction in species-richness with increased nitrogen availability has been associated with dominance by fast growing species and grasses, which out-compete other species. Moreover nitrogen fixing species lose their competitive edge when the soil-nitrogen availability is increased (Mountford *et al.*, 1993).

The above observation may be supported by comparing the forb and graminoid distribution along the water-regime and nitrogen availability (Figure 4.13). The gradient showed that graminoids dominate over forbs at higher nitrogen availability or wetter regions (Olde Venterink *et al.*, 2001a). The dominance of graminoids at higher nitrogen availability is due to their vigorous competitive ability, especially considering their rapid growth (Pywell *et al.*, 2003) and shading over of other species (e.g. Rebele, 2000; Joyce, 2001). The point at which the switch between graminoid and forb distribution occurs *i.e.* beyond water-table depth of 55 cm, coincides with the point where the soil-nitrogen availability decreases significantly to 40 µmol.

Retrospective examination of soil conditions from the established community properties was done through Ellenberg scores. Mean weighted Ellenberg scores for moisture (F) calculated from the existing species correlated well with the field soil water-regime and soil-nitrogen availability (Figure 4.11). On the other hand, mean weighted Ellenberg scores for soil-nitrogen (N) did not show any relationship with soil moisture level or soil-nitrogen availability (Figure 4.12). This second observation is in line with the identified weakness of Ellenberg scores for studying soil chemical properties (Schaffers and Sykora; 2000).

4.4.5 Canonical Correspondence Analysis

Canonical Correspondence Analysis showed how the species relate along multiple axes of plant characteristics and environmental gradients (Figure 4.14). The analysis of the species distribution showed an appreciable difference between the species used for the mesocosm study (Chapter 3) namely *C. nigra*, *F. pratensis* and *S. officinalis*. *Festuca pratensis*, was found in regions of drier soil and higher soil-nitrogen availability while *C. nigra* was at a region of moist soils but of lower nitrogen availability. *Sanguisorba officinalis* was found ordinated in drier regions but associated with quadrats of higher tissue nutrient concentration. Overall tissue nutrient concentrations did not show a direct response to gradients of soil water-regime or soil-nitrogen availability as investigated earlier. Therefore this may itself be a product of community composition. Nevertheless, integrating this tissue concentration data with environmental variables like soil water-regime and nitrogen availability helps in understanding species preferred zones and may be useful as a potential tool in formulating conservation / restoration strategies (Boeye *et al.*, 1997).

4.4.6 Involvement of nitrogen availability on plant response

The results from this study suggest that plant response to water-regime may be mediated by nitrogen availability. In support of this the following may be considered. Firstly the site is well supplied with water, which indicates water availability *per se* should not be solely behind plant response (Davies and Gowing, 1999); suggesting that other resources associated with water-regime might be playing a role (*sensu* Goldberg, 1990). Secondly, a significant relationship between soil water-regime and nitrogen availability was observed (Figure 4.6). Third, from the vegetation analysis (*i.e.* nitrogen to phosphorus ratio), it was found that the species in the site were limited with nitrogen. Fourth, soil-nitrogen availability showed significant influence on plant biomass production, tissue nutrient concentration and species-richness.

However, step-wise regression suggested water-regime was stronger than soil-nitrogen availability in explaining the plant response. This could be due to other water-regime related factors, for e.g. plant response to aeration stress. Also the number of different species present (from grasses and legumes to sedges and rushes within the study) means an array of differences in physiological adaptation in demand and strategy of acquiring nutrients (Sorrell *et al.*, 2000; Van Duren and Pegtel, 2000). This consequently could have played a role in weakening particularly the nutrient relationship as well as distribution of the three species from this study (See Figure 4.15). Competition for other factors like light may also modify the plant response to nutrient availability and water-regime (Rebele, 2000; Kotowski, 2002). Further elaboration of these factors in the future may help better clarify the relationships between soil water-regime, soil-nitrogen availability and plant response.

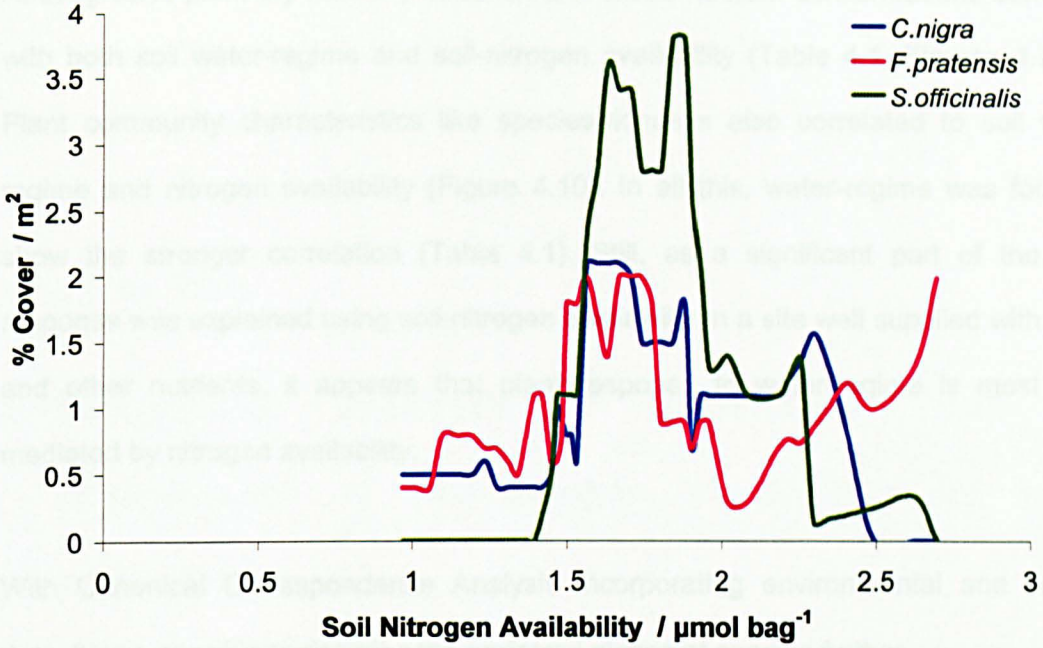

Figure 4.15 Distribution of *F. pratensis*, *C. nigra* and *S. officinalis* along median soil nitrogen availability, between April 28th-June 9th, 2003 as rolling average (n=44)

4.5 Conclusion

Soil-nitrogen availability varies along gentle gradients in soil water-regime. Maximum nitrogen availability was recorded at a range considered to be free from soil aeration and drying stress for the soil type, as specified through plant growth thresholds (Gowing and Spoor, 1998).

Plant distributions showed a switch from one species to another over a range as narrow as 10 cm (Figures 4.4, 4.13). Similarly, plant species distribution correlated with both soil water-regime and soil-nitrogen availability (Figures 4.4, Figure 4.13).

Aboveground plant dry matter production and tissue nutrient concentrations correlated with both soil water-regime and soil-nitrogen availability (Table 4.1, Figures 4.7-4.9). Plant community characteristics like species-richness also correlated to soil water-regime and nitrogen availability (Figure 4.10). In all this, water-regime was found to show the stronger correlation (Table 4.1). Still, as a significant part of the plant response was explained using soil-nitrogen availability in a site well supplied with water and other nutrients, it appears that plant response to water-regime is most likely mediated by nitrogen availability.

With Canonical Correspondence Analysis incorporating environmental and harvest data, it was possible to describe the preferred niches of species further.

Chapter Five

5 Integration: influence of water-regime and the involvement of soil-nitrogen availability on plant response

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This chapter summarizes the experimental findings from the past three chapters. It also presents results of additional experiments conducted.

5.1 Introduction

Subtle differences in water-regime are an important force in structuring plant communities (Silvertown *et al.*, 1999). In this context, this thesis investigated whether these differences in water regime, particularly in soils where water is freely available (Davies and Gowing, 1999), are mediated through other associated changes for e.g. in soil chemistry (Neill, 1990). In such situation, water supply is an indirect gradient (*sensu* Goldberg, 1990), and therefore the search for a resource involved in plant response becomes valid. One such resource significantly linked with differences in water regime is nitrogen (Stanford and Epstein, 1974). Specific experiments to elucidate the involvement of nitrogen in plant response were then conducted at laboratory, mesocosm and field levels. See flow chart in Figure 5.1.

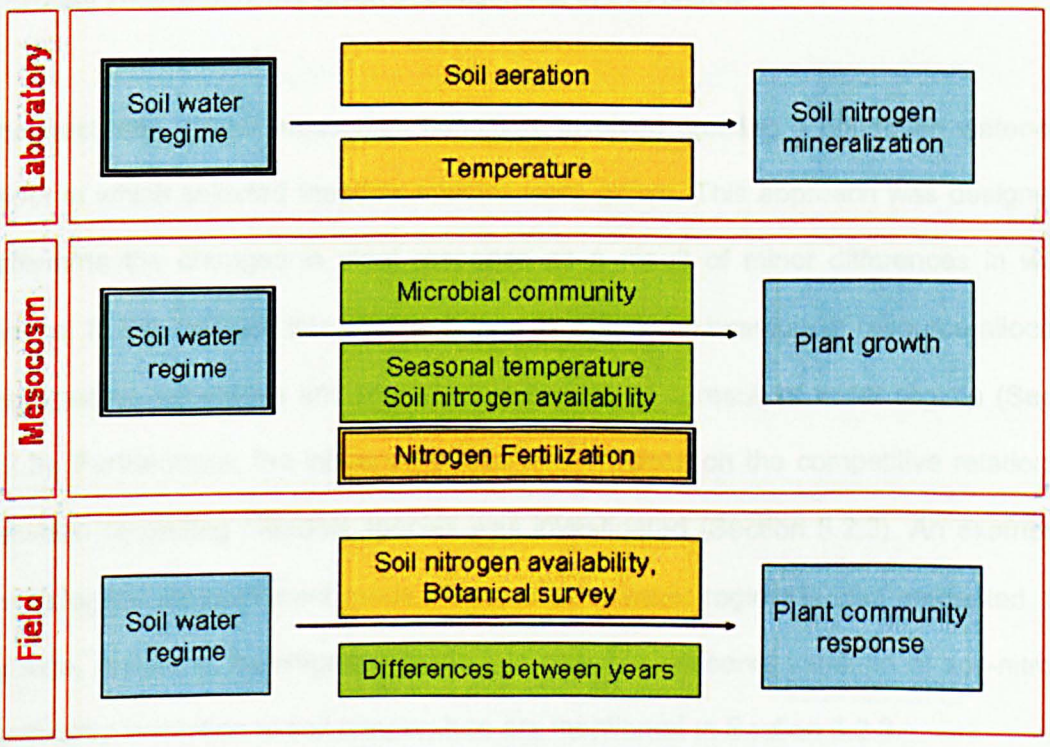

Figure 5.1 Thesis outline flowchart. New experimental results incorporated in this chapter are highlighted in green. Double bounded boxes indicate controlled variables.

The first experiment, conducted in the laboratory, sought to establish the dependence of the resource – *i.e.* nitrogen availability on water-regime. In this experiment, the aim was to elaborate to what extent nitrogen mineralization was influenced by minor variations in water-regime (Section 2.4.1 Chapter 2). The results from this question opened another line of investigation: how does water regime influence nitrogen mineralization? This led to the study of the influence of soil aeration on nitrogen mineralization (Section 2.4.3 Chapter 2). After successfully elaborating the involvement of aeration the next step addressed was: are the above related to changes in microbial community composition? This was particularly inspired by suggestions from earlier studies e.g. Hacin *et al.* (2001) who suggested a shift in microbial community composition may be involved. This study was then aimed to bolster the observations from the earlier chapters by directly linking with the microbial mediators. Details of microbial involvement are given in Section 5.2 of this chapter.

The next step, under mesocosm condition, involved creating a controlled water-table depth in which selected meadow species were grown. This approach was designed to determine the changes in plant response as a result of minor differences in water-regime. In this respect, this chapter brings an additional example of resource allocation response on vegetative and reproductive tissues as a result of water regime (Section 5.2.3). Furthermore, the influence of this water regime on the competitive relationship between coexisting meadow species was investigated (Section 5.2.3). An example of physiological measurement made in response to water regime is also presented. Side by side, results of investigation made into possible seasonal variation of soil-nitrogen availability in relation to soil temperature are mentioned in Section 5.2.3.

On a field level, the study monitored *in situ* soil-nitrogen availability as a result of the various water-table depths achieved. In addition to observing relationship between plants biomass production and nutrient conditions, this section describes the variation in plant distribution within years differing in precipitation and hence water regime (Section 5.2.6).

Lastly, a general compilation of results with respect to the research questions are presented in the following sections. Specific results of the additional experiments are presented within the main sections.

5.2 Minor differences in water-regime create differences in nitrogen availability

In natural unfertilized ecosystems, nitrogen mineralization is main source of plant available nitrogen (e.g. Abbasi *et al.*, 2001). Soil moisture is one of the major factors influencing soil-nitrogen mineralization and availability (e.g. Stanford and Epstein, 1974).

A survey of literature on nitrogen mineralization from laboratory incubated soils showed a depression of nitrogen mineralization at soil moisture regimes near saturation except for the meadow soil with roots (Chapter 2, section 2.4.1). A slower decline in mineralization as the soil dries was also evident. In this thesis experiments conducted at laboratory, mesocosm and field scales also showed a similar response (Chapter 2, section 2.4.1; Chapter 4, section 4.3.2; this chapter section 5.2.2 Figure 5.4). Further experiments revealed the key controlling factor of mineralization in moist soils was

aeration. Mineralization was limited when the soil air-filled pore space declined below 10% (Chapter 2, section 2.4.3). As mineralization is known to be a microbial mediated process, this lead to the question: could this response in nitrogen mineralization be related to changes in soil microbial community? This is investigated in the following section.

5.2.1 Role of microbial community

The difference in mineralization due to soil water-regime and aeration is likely to be a result of a shift in soil microbial community. This has been particularly suggested in waterlogged soils (Tusneem and Patrick; 1971; Patrick, 1982 in Wang *et al.*, 2001). A number of micro organisms are involved in nitrogen mineralization. The most common of these organisms are given in Table 5.1. Any factor thus influencing these micro organisms e.g. soil moisture or aeration is thus expected to affect nitrogen mineralization. Soil moisture when sufficiently available has its main effect by controlling aeration via gas diffusion (Grant and Rochette, 1994). This effect of soil aeration on nitrogen mineralization depends on whether the mineralizing organisms are aerobic or anaerobic. In general, most soils show a reduction in mineralization rate when there is poor aeration (Lewis, 1986).

Table 5.1 Some typical soil organisms involved in nitrogen mineralization by category (after Lewis, 1986)

Ammonifying bacteria	Actinomycetes	Fungi
Pseudomonas spp.	*Streptomyces spp.*	*Alternaria spp.*
Bacillus spp.		*Aspergillus spp.*
Clostridium spp.		*Mucor spp.*
Serratia spp.		*Pencillium spp.*
Micrococcus spp.		*Rhizopus spp.*

Microbial community composition in response to subtle differences in water-regime was monitored using a Phospholipids Fatty Acid (PLFA) technique. This technique reveals the presence and abundance of particular organisms or groups of organisms by identifying specific cell membrane fatty acid signatures (Frostegard *et al.*, 1993; Hill *et al.*, 2000). The PLFA study was undertaken according to Pawlett, 2004). PLFA signature were studied on mesocosm soils subjected to water-table depths of 5, 15, 25, 35 and 45 cm. Thirty-eight PLFA groups were identified (See Appendix 4). From this, PLFAs which correlated significantly (p=0.05) with the water-table gradient were selected by step-wise regression. These 20 significant PLFAs were then ordinated on covariance using Principal Component Analysis. The result is shown in Figure 5.2.

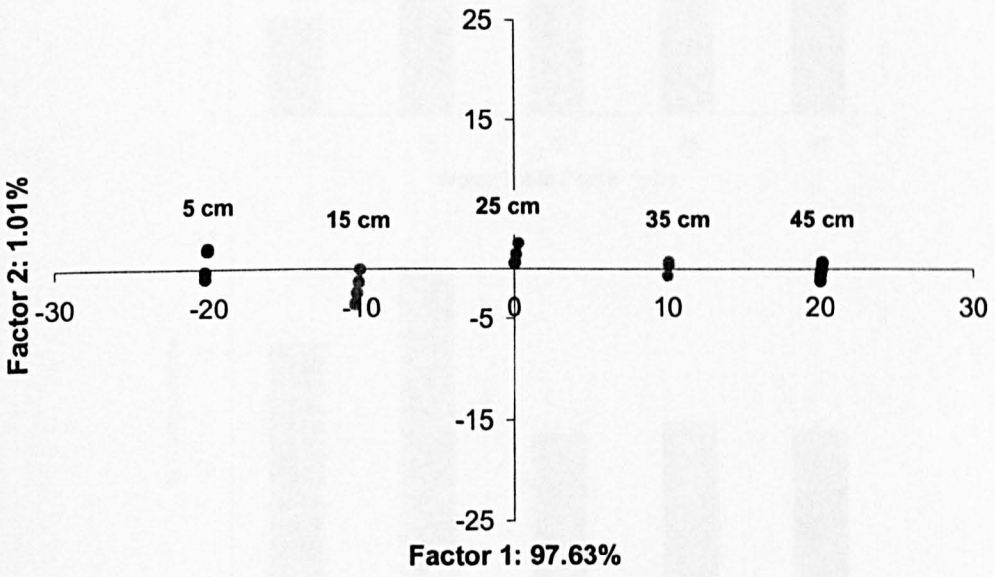

Figure 5.2 Principal Component Analysis ordination of soil microbial community composition from PLFA study. Water-table depths are shown above the individual points. Factor 1 appears to strongly correlate with water-table depth (n = 4 samples). The percentage shows the degree of variation explained by the respective axes.

The response of specific micro-organism community components could also be investigated from the results (see Figure 5.3). For example, specific biomarker fatty acid of mycorrhizal fungi was 16:1 ω 5 and 10-Me 17:0 for actinomycetes. Mycorrhizal fungi responded positively to soil drying (r^2 = 0.65, $p < 0.001$), indicating that mycorrhizal fungi do better at the drier end of the water-regimes tested. However, actinomycetes did better as the soil became wetter (r^2 = 0.41, $p< 0.01$).

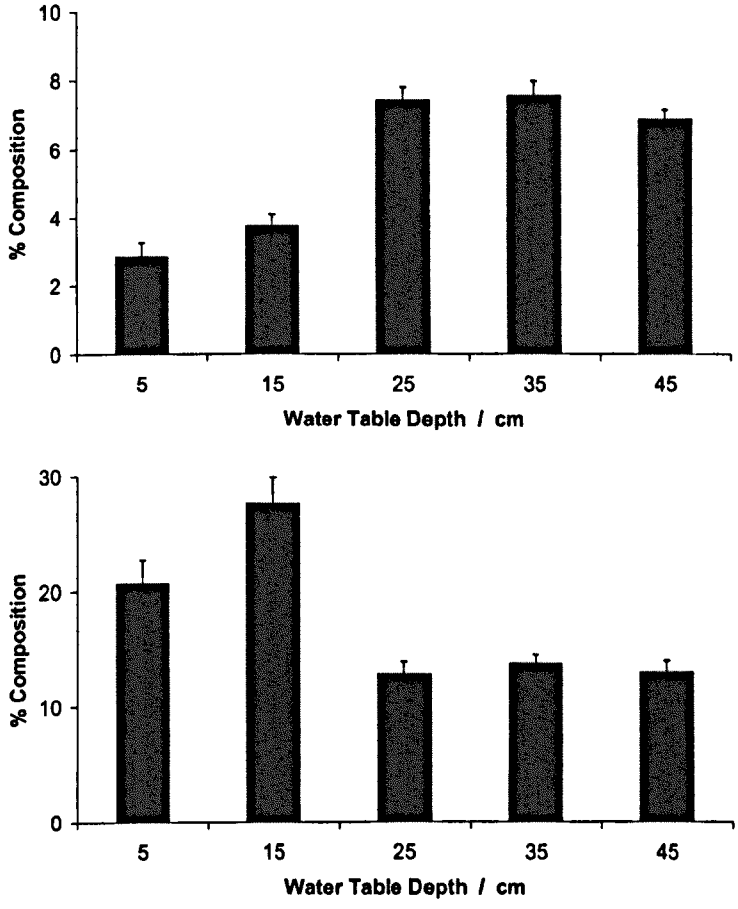

Figure 5.3 Response of selected microbes (a) mycorrhizal fungi and (b) actinomycetes to water-table depth. % composition within the overall microbial community is presented. Bars denote standard error.

The above observation on the presence of mycorrhizal fungi in the soil was also corroborated by direct infection examination on the roots of plants growing in these mesocosms. The examination method involved clearing roots using hot potassium hydroxide (1 M KOH) and staining with trypan blue before examination under a light microscope (Sylvia, 1994). Signs of infection, namely hyphae and arbuscules were sought for. The infection was then assessed in terms of relative scale: rare, occasional, frequent and abundant. A sample slide with arbuscules is shown in Figure 5.4. From this examination, evidence of mycorrhizal infection was found for *F. pratensis* but not for *C. nigra*. Overall results and the number of slides examined are shown in Table 5.3.

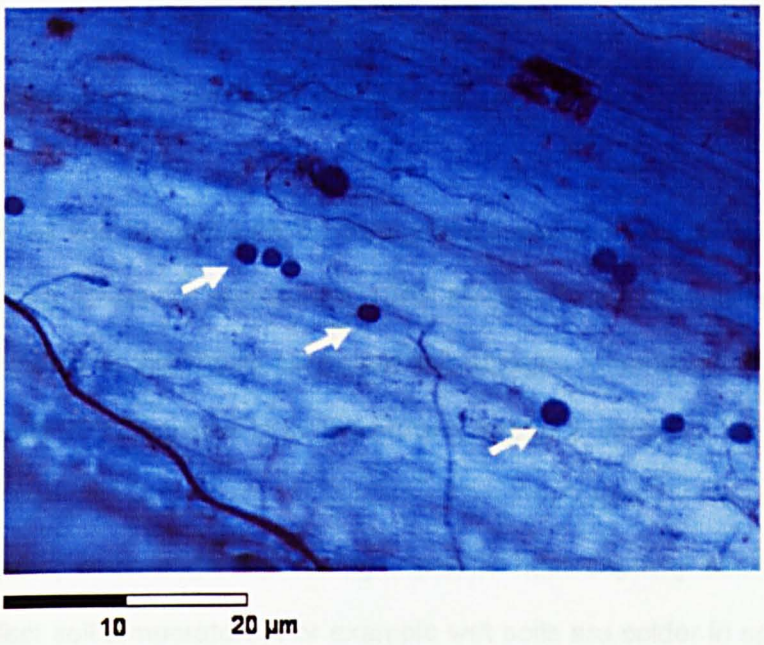

Figure 5.4 Mycorrhizal vesicles on the root *F. pratensis* stained with trypan blue (×200 magnification). Arrows point some vesicles.

Table 5.2 Presence of mycorrhizal infection on *F. pratensis* roots. Presence is noted per slide examined.

Water-table depth / cm	Number of slides with presence of mycorrhizal infection / relative scale					% slides present
	Rare	Occasional	Frequent	abundant	none	
5	-	-	-	-	4	0
15	-	-	-	-	3	0
25	1	-	-	-	3	25
35	1	1	-	-	3	67
45	2	-	1	-	2	60

5.2.2 Soil temperature, soil water-table depth and nitrogen availability

The influence of soil temperature on nitrogen mineralization is a well documented phenomenon (e.g. Cassman and Munns, 1980; MacDuff and White, 1985; Leiros *et al.*, 1999). Experiments in this thesis confirmed soil-nitrogen mineralization approximately doubles for every 10°C increase in temperature (Chapter 2, section 2.4.4).

On another note, since water has a high specific heat capacity soil wetness could potentially affect soil temperature. For example wet soils are colder in spring than drier soils. Hence the question of how much differences in soil water-regime regulate soil temperatures and the potential effect on mineralization was tested. For 9 months from October 2003 to June 2004, soil temperature in mesocosms was recorded hourly using thermocouples buried at 5 cm below the soil surface. The result of this monitoring is shown in Figure 5.5. During this time, soil-nitrogen availability was also monitored using

ion-exchange resin bags (see Chapter 4, section 4.2.3) which were renewed every 2 months.

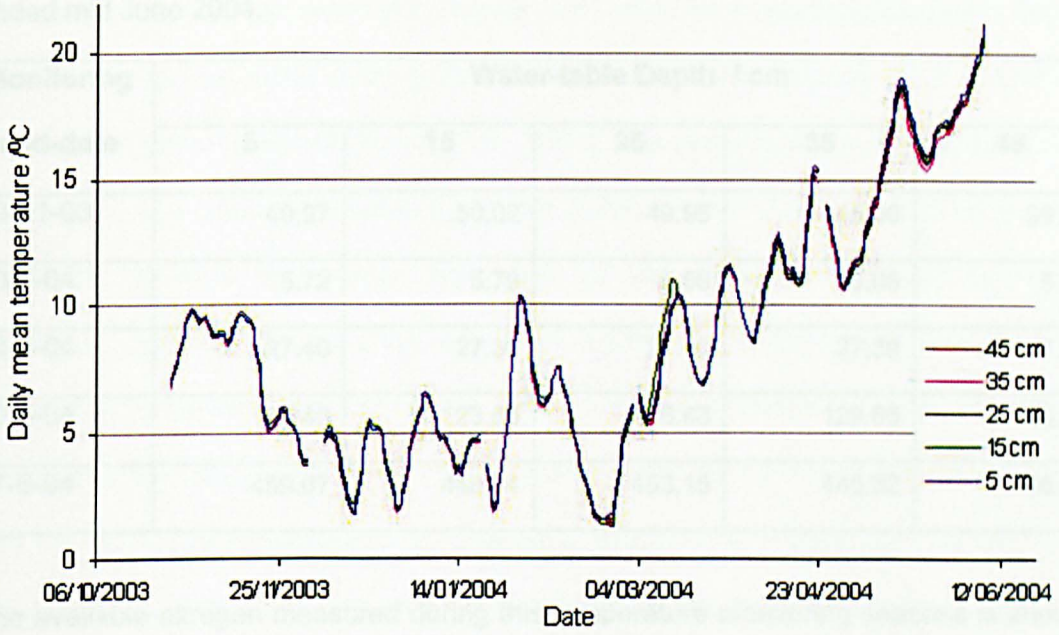

Figure 5.5 Soil temperature monitoring data in mesocosms with water-table depths of 5, 15, 25, 35 and 45 cm. n=4 for each water-table depth. The daily mean temperature is calculated as an average of the daily maximum and minimum temperatures.

The temperature data were then summarized using the degree-day concept (Snyder, 1985) where the mean of the maximum and minimum daily temperature is calculated compared to a threshold temperature of 5 °C (Broad and Hough, 1982). The degrees in excess of the threshold are then cumulated over a season. The cumulated mean degree days for the five water-table depths are shown in Table 5.3. Analysis of variance showed that there was no significant influence of water-table depth on soil temperature at any time of the year.

Table 5.3 Cumulated Degree days for mesocosms at different water-table depths. Dates denote end of monitoring time. Monitoring was started mid October 2003 and ended mid June 2004.

Monitoring end-date	Water-table Depth / cm				
	5	15	25	35	45
20-11-03	49.97	50.02	49.95	45.00	39.60
20-1-04	5.72	5.79	5.66	5.66	5.61
28-2-04	27.40	27.30	27.40	27.39	27.21
22-4-04	137.49	123.55	135.63	129.65	130.46
07-6-04	459.07	448.94	453.15	440.52	446.39

The *available nitrogen measured during* this temperature monitoring seasons is shown in Figure 5.6.

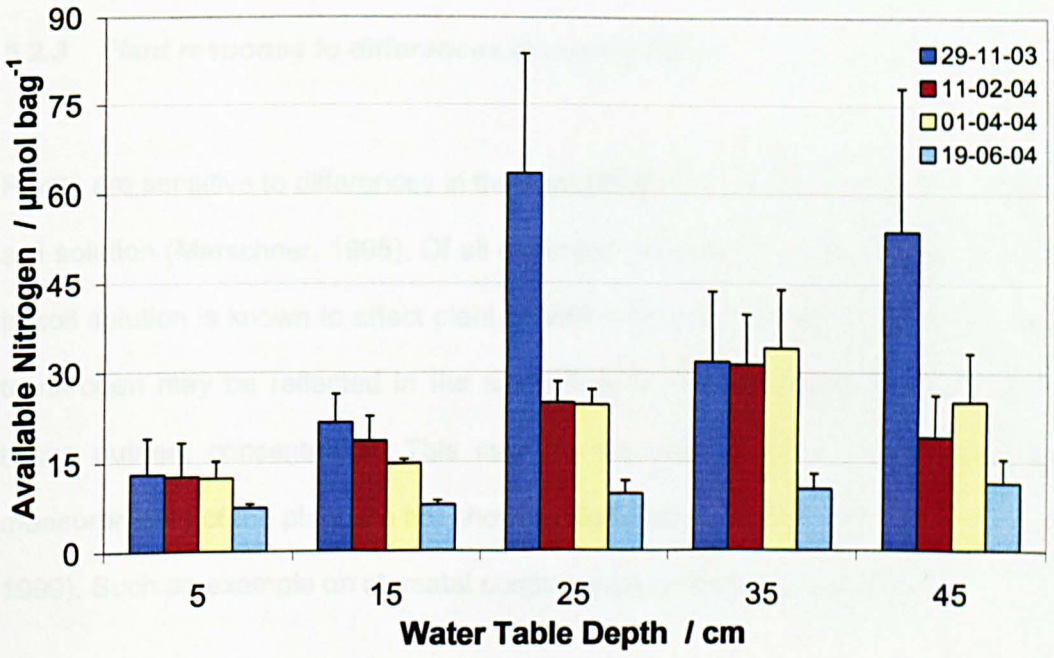

Figure 5.6 Available soil-nitrogen during monitoring season. Dates denote end of monitoring time. Monitoring was started mid October 2003 and ended mid June 2004.

The highest amount of available nitrogen was recorded in late November and the least in June. The former could be attributed to actively mineralizing litter which was put to compensate for the harvested plant matter. This could be a result of the applied litter as well as the lack of active plant growth during this season meaning more mineralized nitrogen remains in the soil (Hacin *et al.*, 2001). The same reasoning could be used to explain the low amount of available nitrogen in June, when plants are actively growing. However, analysis of variance performed on the nitrogen availability during the monitoring seasons did not show significant influence of water-regime. This could be partly due to the high variability within the mesocosm replications. On the other hand, the absence of depression of nitrogen availability even at high water-table depths i.e. 5 cm (cf. laboratory mineralization in Chapter 2, section 2.4.1), could also be due to the presence of plants, which act as a conduit for the passage of air from the atmosphere to the soil (Callaway and King, 1996) thereby improving conditions for mineralization.

5.2.3 Plant response to differences in water-regime

Plants are sensitive to differences in the availability and concentration of nutrients in the soil solution (Marschner, 1995). Of all essential nutrients, the concentration of nitrogen in soil solution is known to affect plant growth most (Marschner, 1995). Plant response to nitrogen may be reflected in the amount of dry matter produced, its allocation or tissue nutrient concentration. This may be the case even if external physiological measurements of the plant are not showing significant differences (Davies and Gowing, 1999). Such an example on stomatal conductance is shown in Figure 5.7.

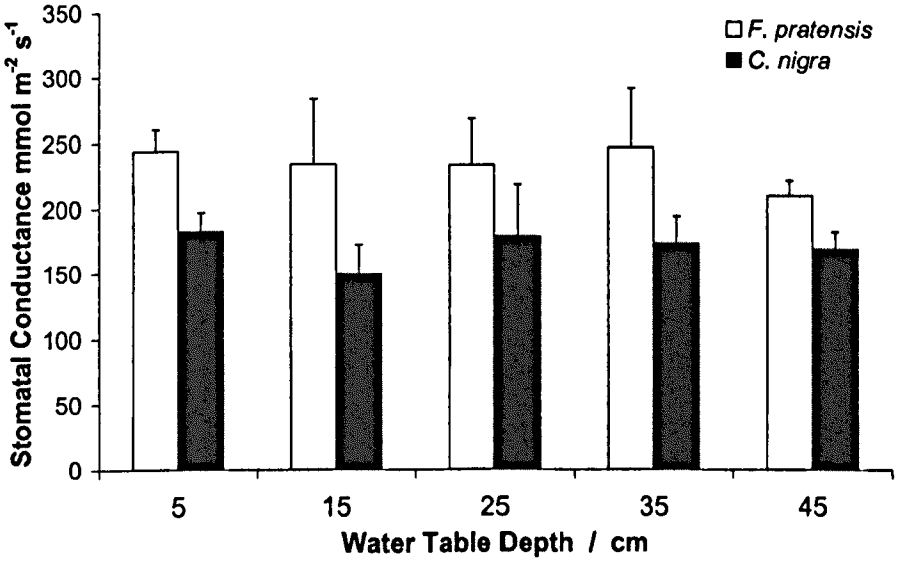

Figure 5.7 Stomatal conductance of *F. pratensis* and *C. nigra* at water-table depth treatments of 5 - 45 cm. Bars denote standard error.

On an individual species level, in the mesocosm study, plant dry matter production responded to differences in soil water-regime and that of soil-nitrogen availability. Plant response in monoculture across a gradient of water-table depths of 5 – 45 cm showed maximum aboveground biomass production and nitrogen uptake at 15 cm (Chapter 3, section 3.4.1). This is below the 25 cm where maximum soil-nitrogen mineralization was recorded in the laboratory. A possible explanation for this observation could be due to passive plant aeration of the soil media by acting as a conduit to oxygen (Callaway and King, 1996; Sorrell *et al.*; 2000) thereby improving the growing conditions in the rhizosphere. On the other hand, dry matter production was also increased by the application of nitrogen fertilizer (Chapter 3, section 3.4.5). The yield increase due to fertilization was more pronounced at the drier i.e. 45 cm water-table depth than at the 5 cm water-table depth. This may be because of denitrification which occurs under the

wet soil conditions where water-filled pore space exceeds 60 – 80 % (Machefert and Dise, 2004).

Plant resource allocation varied along the water-regime gradient. Root to shoot ratio for *F. pratensis* differed between water-table depth treatments (Chapter 3, section 3.3.2.3). More root biomass was produced at the wetter end of the soils for both species (Figure 5.8). Tilman (1988) suggests that more allocation to the resource capturing part, in this case of the roots, is a response to the development of stress. According to Reynolds and D' Antonio (1996) a resource most likely associated with such response is nitrogen.

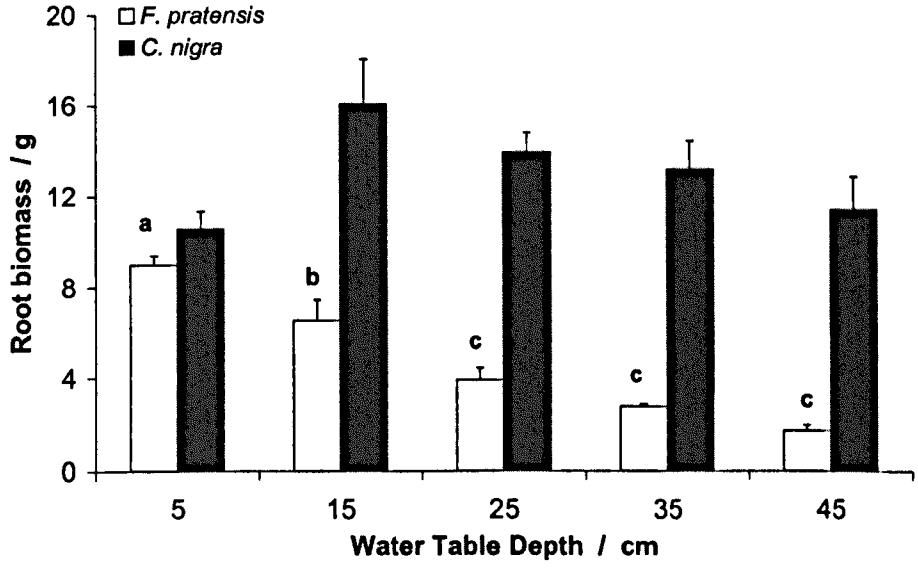

Figure 5.8 Root biomass productions for *F. pratensis* and *C. nigra* in response to differences in water-table depth (monoculture). Letters a, b refer to the ranking of biomass production. Bars denote standard error.

On the other hand, resource allocation for vegetative and reproductive tissue of *F. pratensis* responded significantly ($p < 0.05$) to the water-regime gradient (Figure 5.9). Drying encouraged more allocation of resource to the reproductive tissue, while wetting

encouraged vegetative allocation. The maintenance of reproductive tissue production at the expense of vegetative tissue is a known response of plants to water-deficit stress (Bray, 2000).

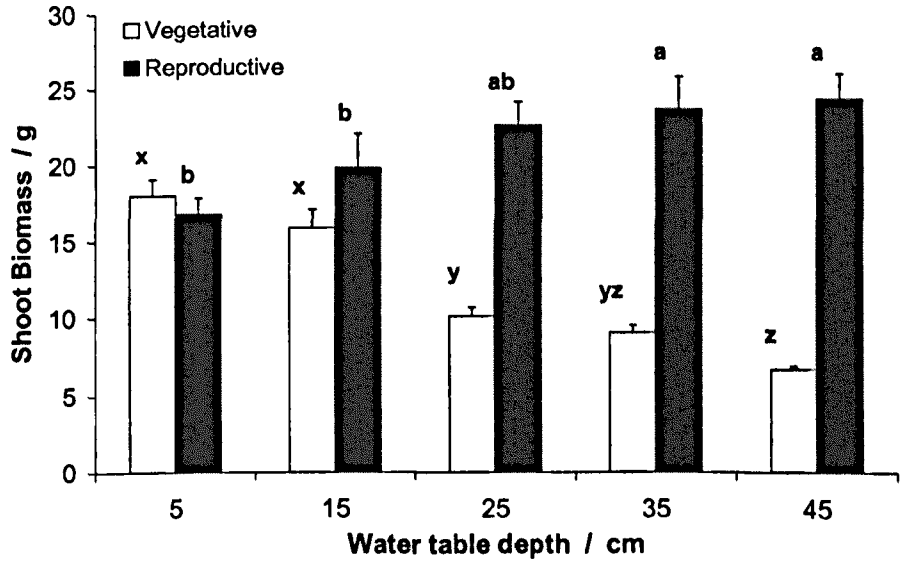

Figure 5.9 *F. pratensis* shoot biomass allocation in response to differences in water-table depth (monoculture). Letters a, b & c refer to the ranking of reproductive tissue, while x, y, z for vegetative. Bars denote standard error.

5.2.4 Plant competitive response to differences in water-regime

When plants are grown in close proximity, they exhibit competition for the common resources needed for their growth (Grime, 2001). Interspecific competition between *F. pratensis* and *C. nigra* along a gradient of soil water-table depth and nitrogen availability was studied here.

The results showed that even under the same environmental conditions, plant responses are very different when grown in monoculture or mixture with other species

(Figure 5.10a and b). The differences between the responses of the two species may be illustrated using the *Festuca pratensis* to *Carex nigra* biomass ratio under monoculture and mixture (Figure 5.11). The *F. pratensis* to *C. nigra* ratio in monoculture does not vary across the water-table depth treatments. This indicates both species respond similarly to the environmental conditions in monoculture. However in mixture the ratio shows a 7 fold difference between the two, especially at the drier water-table depths.

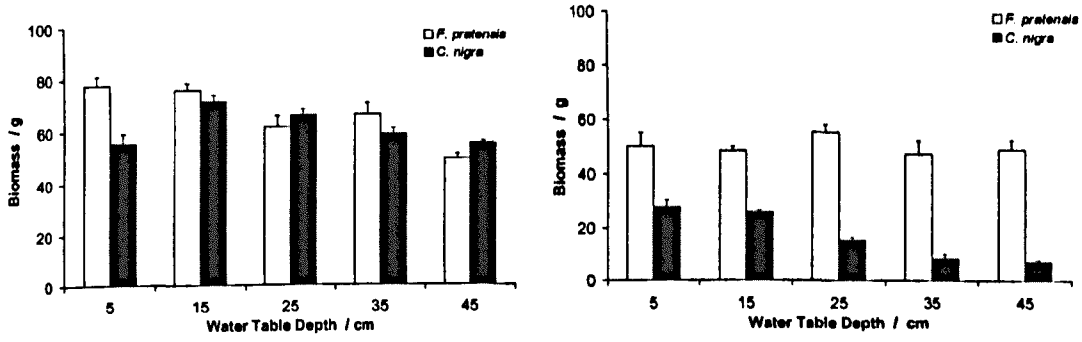

Figure 5.10 *F. pratensis* to *C. nigra* nitrogen biomass production ratio when grown in monoculture (a), and mixture (b) at different water-table depths.

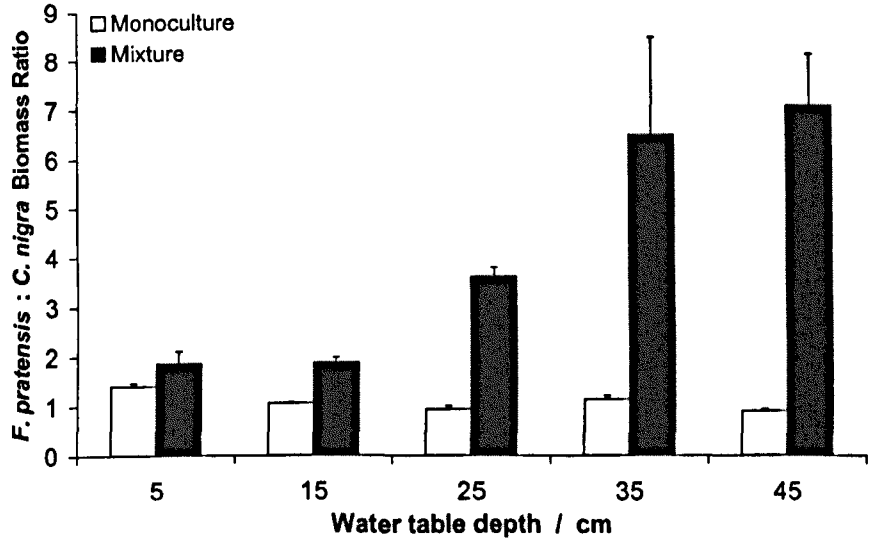

Figure 5.11 Ratio of *F. pratensis* to *C. nigra* biomass production when grown in monoculture and mixture at different water-table depths. Bars denote standard error.

On the other hand, even though the difference in biomass production between *C. nigra* and *F. pratensis* is strong as shown in the above figure, the combined total biomass production of the two species in monoculture and mixture still remains the same (Figure 5.12).

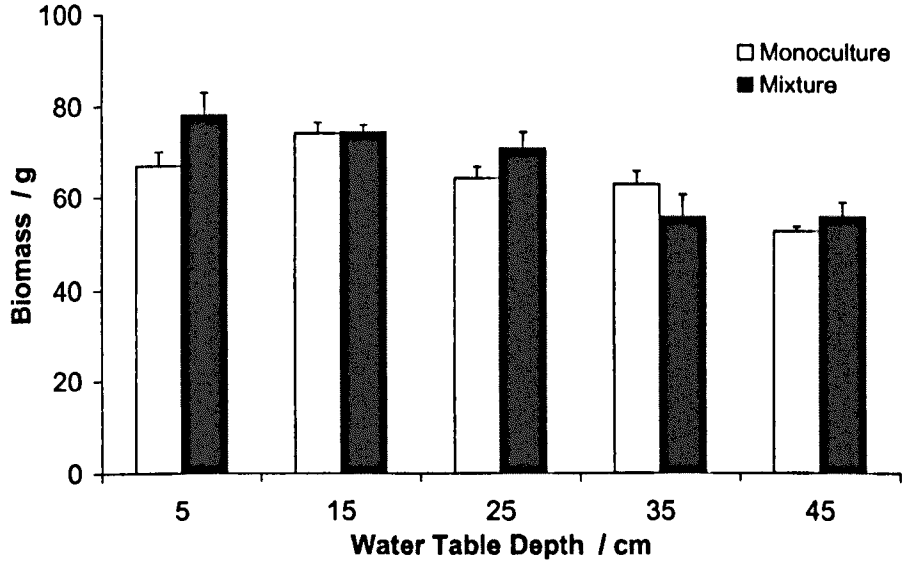

Figure 5.12 Combined biomass production of *F. pratensis* and *C. nigra* in monoculture and mixture at different water-table depths. Bars denote standard error.

Overall, the observations show that under wet soil conditions *C. nigra* can survive as the competitive edge of *F. pratensis* is suppressed.

5.2.5 Involvement of nitrogen availability in plant response to water-regime

The experimental setup in the mesocosm study ensured the only limiting resource was nitrogen. Further test on the involvement of nitrogen in the response was made by a

fertilization study (Chapter 3, section 3.4.5). Fertilization using nitrogen showed an increase in biomass production, hence confirming this limitation.

An increased availability of soil nitrogen meant that the main influence of water regime on biomass production (*C. nigra*) or tissue nitrogen concentration (*S. officinalis*). was cancelled out (Section 3.3.4 Table 3.14). Moreover fertilization that supplements soil-nitrogen mineralization showed negation of the influence of water-table depth on the competitive abilities of *C. nigra* and *S. officinalis* (Section 3.4.5) This happened to both species, which have contrasting preferred regions of water-table depth (as shown in Chapter 4, section 4.3.1). For both species and both water-table depths, fertilization reduced the competitive ability of the species originally suited to the preferred water-regime while it improved the competitive ability of the disadvantaged species. This response is likely to get stronger with the passage time.

Further evidence supporting the involvement of nitrogen availability in mediating plant response to water-regime comes from studies conducted on pure sand media where nitrogen was externally supplied. Such an arrangement in effect removes mineralization being the sole source of available nitrogen. This explains why under such conditions no influence of water regime is observed in plant response, as shown in the following studies. A study conducted by Güsewell *et al.*, (2003) on a number of wetland species, with water-table depths of 3 to 23 cm, found that water-regime did not have significant influence on plant response. Similarly, Lodge *(in prep.)* found that water tensions of 30 and 190 cm did not show any significant effect on the biomass production of *Carex disticha.* This is in contrast to the findings here where *F. pratensis* and *C. nigra* differed in biomass production and tissue nitrogen concentration with water-table depths varying by just 10 cm on a range of 5 – 45 cm.

Some examples on the negation of the influence of water-regime upon fertilization on plant competitive ability are given as follows. A study by Neill (1990) on whitetop grass *Scolochloa festucacea* and cattail *Typha glauca* found that a reversal of species biomass production between shallow and deep water levels was reversed by fertilization with nitrogen. Similarly, Berendse and Aerts (1984), studied competition between *Molinia caerulea* and *Erica tetralix* at water-table depths of 0, 20 and 40 cm with nitrogen fertilization. They found that at wetter soil conditions *i.e.* water-table depths of 0 and 20 cm, fertilization reduced the competitive advantage of *M. caerulea* against that of *E. tetralix*. Levine *et al.* (1998) also documents a nutrient-induced reversal of the competitive dynamics of salt marsh perennials at ambient marsh conditions.

On community level, the field monitoring of a species-rich meadow found increase of dry matter production with increase in soil-nitrogen availability (Section 4.3.3 chapter 4) and reduction in species richness (Section 4.3.4). Manipulative fertilization experiments conducted in meadows also show similar response, but more pronounced (e.g. Mountford *et al.*, 1993; Kirkham and Wilkins, 1994b). This response could be of short term effect i.e. when increased nutrient availability encourages more biomass production (e.g. Kirkham and Wilkins, 1994b) or of long term significance, with the competitive exclusion of less productive species (e.g. Kirkham and Wilkins, 1994a; Joyce, 2001). In time fertilization then results in reduction of species richness (e.g. Smith *et al.*, 2000, Pywell *et al.*, 2002).

5.2.6 *Plant community properties response to water-regime and nitrogen availability*

Plant distribution along a hydrologic gradient is a well documented observation in wet meadows (e.g. Ellenberg, 1988; Gowing and Spoor, 1998; Dwire *et al.*, 2004). Quantitative data on the preferred plant habitation zones in wet meadows at minor differences in water-regime have also been shown by Gowing *et al.* (2002). Interestingly, shifts of whole plant community composition upon differences of water-regime (as a result of annual weather) have also been found (Gowing *et al.*, 2002). Using two year plant community composition data (2000 and 2002) from a species-rich meadow, the shift in the abundance of the three species studied in this thesis: *F. pratensis*, *C. nigra* and *S. officinalis* is shown in Figure 5.13.

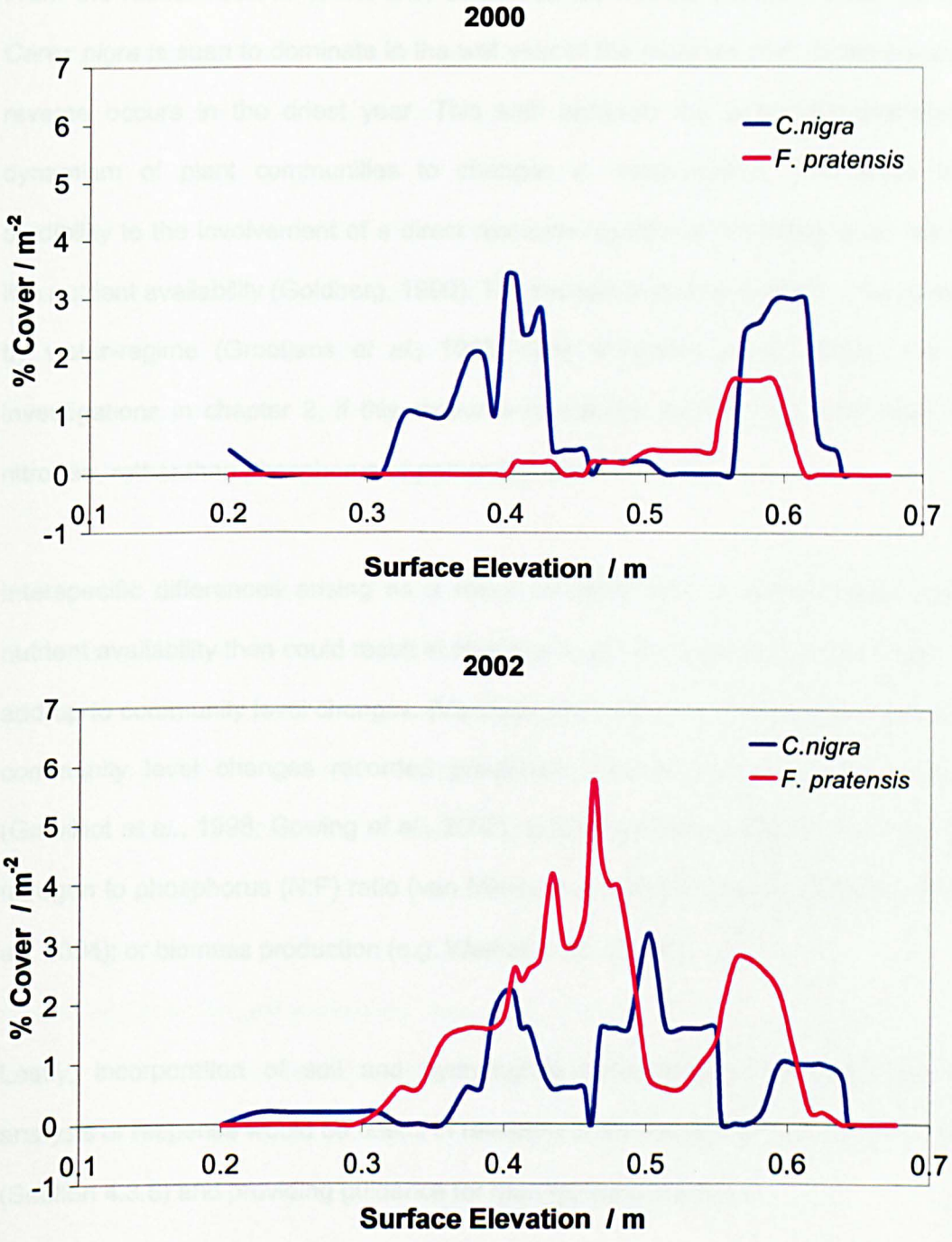

Figure 5.13 Species distribution of *F. pratensis*, *C. nigra* and *S. officinalis* versus water-table depth for years (2000 and 2002). % Cover rolling average (n = 44). The surface elevation is relative to the lowest quadrat in the site. The elevation dictates the water table depth achieved after flooding.

From the rainfall records of the site, 2000 was the wettest year and 2002 the driest. *Carex nigra* is seen to dominate in the wet year at the expense of *F. pratensis* and the reverse occurs in the driest year. This shift between the years demonstrates the dynamism of plant communities to changes in water-regime. This lends further credibility to the involvement of a direct resource capable of mediating plant response like nutrient availability (Goldberg, 1990). The nutrient that is most likely to be controlled by water-regime (Grootjans *et al.*, 1985; Olde Venterink *et al.*, 2002). And from investigations in chapter 2, if this resource is a major nutrient it is most likely to be nitrogen, rather than phosphorus or potassium (Section 2.4.5).

Interspecific differences arising as a result of differences in water regime and soil nutrient availability then could result in changes in species distribution which later could add up to community level changes. (Vermeer and Berendse, 1983). Examples of such community level changes recorded previously include: in community composition (Grevilliot *et al.*, 1998; Gowing *et al.*, 2002), in plant nutrient concentrations like tissue nitrogen to phosphorus (N:P) ratio (van Mierlo *et al.*, 2000), species-richness (Dwire *et al.*, 2004); or biomass production (e.g. Weiher *et al.*, 2004).

Lastly, incorporation of soil and hydrological characteristics for community level analysis of response would be useful in revealing preferred niches of coexisting species (Section 4.3.5) and providing guidance for management practices.

5.2.7 Summary

Understanding how minor differences in water regime influence plant community response was the main objective of this thesis.

The studies conducted in this thesis revealed that, minor differences in soil water regime influence soil nitrogen mineralization and availability. In this response there was a significant influence of water regime on micro organisms which are responsible for nitrogen mineralization. However, the influence of subtle water regime on soil temperature was not found to be significant.

Plants showed a significant response to the influence of water regime in terms of biomass production, resource allocation and competitive interaction. Nitrogen fertilizer application was found to overcome the influence of water regime on species biomass production and tissue nutrient concentration. However, its influence on competitive interaction was not clearly significant with the data from this thesis.

Plant distribution and community response also showed significant response to water regime and soil nitrogen availability. Also better visualization of species and community response was possible by integrating water-regime and soil-nitrogen availability with characteristics of harvested biomass.

Chapter Six

6 Conclusions

The main findings of this thesis on the relationship between water-regime and soil-nitrogen availability and its consequences for plant competition are presented.

Identified ecological drivers controlling the equilibrium of species coexistence in wet meadows include site hydrology, soil nutrient availability and vegetation management. Of these, depth of the water-table has been considered as a primary factor (e.g. Hayati and Proctor, 1990; Van Duren and Pegtel, 2000) and been frequently employed in management decisions (e.g. Oomes *et al.*, 1996; Joyce and Wade, 1998). A mechanistic understanding of how water-regime influences species coexistence is thus vital for guiding conservation practices.

To date few studies have elaborated the influence of minor differences (typically soil matrix potentials of < 5 kPa or water-table depth < 50 cm) in water-regime in structuring wet meadow plant species (e.g. Silvertown *et al.*, 1999). Still fewer have studied the mechanism of how water-regime influences plant coexistence (e.g. Levine *et al.*, 1998). The aim of this thesis was to elaborate the above through studies of: (1) the influence of water-regime on soil-nitrogen mineralization and availability; (2) how differences in soil water-regime and nitrogen availability relate with plant dry matter production and resource allocation; and (3) how those differences modify plant competitive interactions.

These aims were achieved by conducting a three-tier set of investigations: a fully-controlled laboratory experiment investigating the influence of water-regime on soil-nitrogen mineralization; partially-controlled mesocosm experiment, where constant water-table depth was maintained and plants grown in competition, and lastly field monitoring of a species-rich meadow site.

6.1 On soil-nitrogen mineralization and availability

- Laboratory, mesocosm and field studies showed soil-nitrogen mineralization and availability was significantly influenced by water-regime (Section 2.4.1).
- Low soil water tensions showed a significant depression in nitrogen mineralization, while a slower decline was observed at drier soil conditions except for a meadow soil with dead roots (Section 2.4.1). The decline in mineralization at low tensions in the experimental loam soil coincided with air-filled porosity of less than 10 % (Section 2.4.3).
- The difference in nitrogen mineralization as a result of water-regime corresponded with a shift in soil microbial community composition (Section 5.2.1).
- Incubation temperature exponentially increased nitrogen mineralization (Section 2.4.4). However, no significant differences in soil temperature as a result of minor changes in water regime were recorded (Section 5.2.2).

6.2 On plant dry matter production, resource allocation and interspecific competition

- Selected meadow species *Festuca pratensis*, *Carex nigra* and *Sanguisorba officinalis* growing in mesocosm at subtly differing water-table depths of 5, 15, 25, 35 and 45 cm responded with significant differences in dry matter production and nitrogen uptake under both monoculture and mixture conditions. In monoculture the plant biomass production was found to be maximum at the ranges of 15 - 35 cm for *F. pratensis* and 15 - 25 cm for *C. nigra*. In mixture, however, *F. pratensis*

maintained its yield at the drier end *i.e.* > 25 cm while *C. nigra* was found to maintain its yield at the wetter end < 15 cm (Section 3.3.2.1 and 3.3.2.2).

- Within the plant itself, resource allocation between root / shoot tissues as well as vegetative / reproductive tissues varied in response to subtle differences in water-regime under both monoculture and mixture conditions.
- In monoculture, the plant root to shoot ratio was found to decrease as the soil became drier for *F. pratensis*, while it was not significant for *C. nigra* (Section 3.3.2.3).
- Reproductive versus vegetative resource allocation also responded to water-regime for *F. pratensis* (Section 5.2.3, Figure 5.9). An increase in reproductive tissue allocation was observed at the drier end of soil water-regime (*i.e.* >25 cm), while more vegetative tissue was produced at the wetter end *i.e.* <15 cm.
- Application of external nitrogen through fertilization increased dry matter production and tissue nitrogen concentration (Section 3.3.4), showing nitrogen was limiting. The influence of water regime on plant biomass production (*C. nigra*) and tissue nitrogen concentration (*S. officinalis*) was negated when fertilization was made. However during the duration of this experiment, although there were indications, no significant influence of fertilization in modifying plant competitive response along water regime treatments was observed.

6.3 On plant community response

- Plant species distribution responded to a gradient of water-regime both in space and time. Distinct zones of dominance (preference) for coexisting meadow species *F. pratensis*, *C. nigra* and *S. officinalis* were observed (Section 4.3.1). The switch

from one to another species was found to occur over a narrow range of water-table depth. The zones of preference shifted in time as well, depending on the amount of precipitation and hence water-table depths occurring between the years (Section 5.2.6). On the other hand, the distribution of the species along only the gradient of measured soil-nitrogen availability in this data did not specifically discriminate between the three species (Section 4.4.6).

- Soil-nitrogen availability varied with water-regime with maximum nitrogen availability recorded at a narrow range between 40 - 45 cm water-table depth (Section 4.3.2). This zone coincided with a reportedly stress free zone between two earlier established thresholds for soil aeration (<10% air filled porosity) and drying stress (>5 kPa soil water tension). (Section 4.2.2)
- There was significant negative relationship between the community aboveground biomass production and tissue nitrogen concentration with soil water-regime. On the other hand, aboveground biomass production and tissue nitrogen concentration showed a significant positive relationship with soil-nitrogen availability (Section 4.3.3).
- Graminoid and forb species distribution showed significant relationship to both soil water-regime and nitrogen availability gradients (Section 4.3.4, Figure 4.13). The switch in dominance occurred at water-table depths of between 50 – 60 cm, with graminoids dominating at the drier end. This coincided also with soil-nitrogen availability of > 40 μmol bag^{-1} nitrogen.
- Plant species-richness was influenced by both soil water-regime and soil-nitrogen availability (Section 4.3.4). Highest plant species-richness was 34 species m^{-2} and found between water-table depths of 50 - 60 cm. This zone coincided with a zone

where neither grass nor forbs were dominating and available soil-nitrogen was less than 40 μmol bag^{-1} nitrogen.

- Ellenberg's ecological ranking scores of species for soil-nitrogen (N) and flooding (F) were compared with direct measurements of soil-nitrogen and water-regime. Ellenberg's mean flooding score showed significant correlation with both soil water-regime and soil-nitrogen availability. However Ellenberg's mean score for nitrogen did not show any relationship with soil water-table depth nor soil-nitrogen availability (Section 4.3.4)
- Step-wise regression analysis showed soil water regime explained more variation in plant response than soil nitrogen availability (Section 4.3.4 Table 4.1). However soil nitrogen availability was also a significant variable.
- Multivariate Canonical Correspondence Analysis ordination of all existing species along gradients of soil water-regime, nitrogen availability and plant tissue nutrient concentrations indicated the preferred niches of particular species (Section 4.3.5). This information could be interpreted to help guide management practices aimed at the conservation of target species.

6.4 Integrating soil-water-regime, soil-nitrogen availability and plant response

- A number of studies have identified water regime plays a major role in structuring plant communities of wet meadows (e.g. Silvertown *et al.*, 1999). In addition, the influence of water regime on soil processes particularly nutrient availability has been appreciated (e.g. Neill, 1990). In spite of the recognition, relatively little attention has been paid on the mechanism of how water regime influences nutrient

availability with a predictable consequence for the distribution of species across such gradients (Levine *et al.*, 1998). Many early studies along this line had focused on radically altered water regimes (drainage or flooding) or externally fertilized conditions (e.g. Vermeer and Berendse, 1983; Oomes *et al.*, 1996). Relatively few studies have been conducted under controlled conditions, especially water table depth (Van Oorschot *et al.*, 2000). Frequent interaction effect between fertilization and water regime has also meant clear relationships were hard to establish (e.g. Figiel *et al.*, 1995). The conservation of species rich wet meadows primarily requires stable site conditions and controlling nutrient availability (Olde Venterink *et al.*, 2002a). Hence an understanding of the relationship between water regime and soil nutrient availability is imperative, as recent deterioration of a number of species-rich sites has been due to inappropriate hydrological management (Critchley *et al.*, 2003).

- In this context, this thesis tried to establish how water regime influences soil nitrogen availability and how they determine plant competition and distribution. The key findings from this thesis were: clarifying how minor differences in water regime influence soil nitrogen mineralization and availability; the influence of water table depth and involvement of nitrogen availability in plant response and interspecific competition; as well as the involvement of nitrogen availability in community response. The knowledge gained could be a useful management tool for identifying goals and target species in relation to existing edaphic and hydrologic conditions.

6.5 Suggestions for further study

- The mechanistic understanding of soil-nitrogen dynamics gained in this thesis may further be improved and used for producing predictive models of species interaction (*sensu* Tilman, 1990). This may particularly be helpful for ecological restoration, especially when transferring reference ecological information to other sites.
- The mesocosm system developed in this thesis can be a useful tool to conduct further multi-species mixture competition studies. This could be used to investigate outcome scenarios of management practices e.g. water regime and nutrient availability on the response of target species.
- Considering the importance of microbial communities for nutrient transformation, the rapid shift in microbial community composition observed in this thesis could be of potential indicator of ecosystem rehabilitation (*sensu* Harris, 2003; Smith *et al.*, 2003).
- The potential of introducing micro-topographical features with the aim of creating a variety of niches through differences in soil water-regime and soil nutrient availability in fostering species-richness could also be investigated.
- Lastly, similar further studies on the response of other species-rich grassland communities as well as recently restored grasslands on former arable land are welcomed to provide a more complete understanding of soil water, nitrogen and plant distribution relationships.

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8 Appendices

Appendix 1. Compilation of nitrogen mineralization data from literature

Appendix 2. HPLC sample chromatogram for Total Free Amino acids analysis

Appendix 3. Mesocosm study data on biomass, nutrient concentration and fertilization

Appendix 4. Phospholipid Fatty Acid (PLFA) assay data

Appendix 5. Field monitoring data from Cricklade North Meadow National Reserve

Appendix 1. Compilation of soil nitrogen mineralization in response to varying water tensions.

Soil water tension in kPa and the 'names' of the soils used are shown. Mineralized nitrogen data is given in mg kg^{-1}.

Standford and Epstein (1974)

Tension (kPa)	*Parshall*	*Amarillo*	*Cecil*	*Aastad*	*Pullman*	*Minidoka*	*Bearden*	*Kranzburg*	*Barnes*
1500	26	34	22.5	46	57.5	19	58	51	23.5
200	29	36	30	51.5	70	24.5	65	55	29
33	36	39.5	36	82	78	76.5	83.5	67	60
10	38	40	39	85.5	94	60	68.5	71.5	56

Myers et al. (1982)

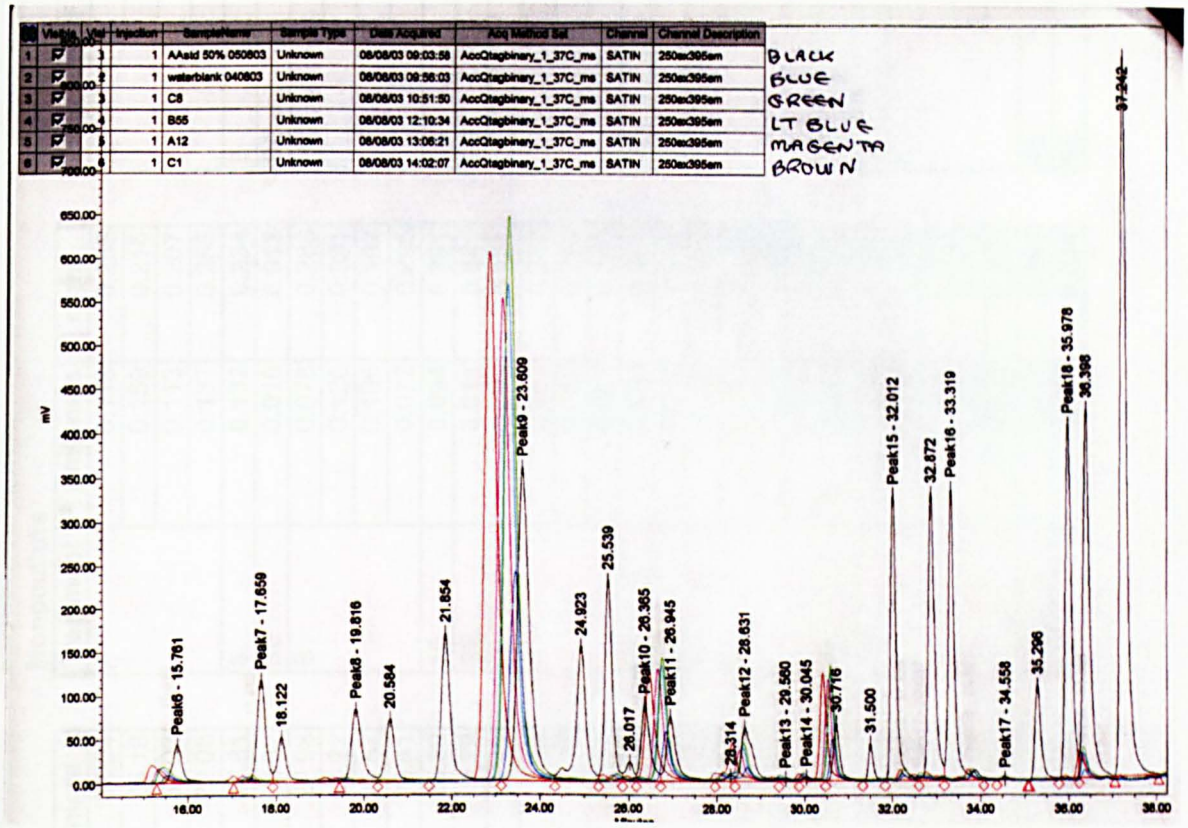
Tension (kPa)	*Narayen B*	*Narayen A*	*Gatton A*	*Gatton B*	*E.T. A*	*E.T. B*	*E.R.E A*	*E.R.E B*	*E.B. A*	*E.B. B*
4000	4	0.5	-3	2	2	4	-2	1.33	5.33	8.66
1500	15	13	4	4	7.5	5.33	-3	0.66	6.66	7.33
500			7.33							
360						6				
340								2		
300					13.5					6.66
200				13.33						
170			17.5							
130	26								10	
100		23					7.5			
50										
33										
30					17	14				
20	30	34	26.5	17.33	19.5		12.5	8.66	12.66	10
10	20	37.5	18.5	18		20	13		12	7.33
5	-6.5	-13	-5	-15.33	-11.5	7.33	-0.5	5.33	-10	-8.66

Sierra (1997)

Tension (kPa)	*Pergamino*
1700	19.25
200	22.4
30	25.9

Appendix 2. HPLC Chromatograms of Total Free Amino Acids (TFAA)

Overlay of chromatographs from a standard, blank extractant and soil samples.



The standard was composed of 25 µmol litre^{-1} of the following amino acids.

Elution time	Amino acid
15.761	Asp
17.659	Ser
18.122	Glu
19.816	Gly
21.854	His
24.923	Arg

Elution time	Amino acid
25.539	Thr
26.365	Ala
26.945	Pro
30.045	Cys
32.012	Tyr
32.872	Val

Elution time	Amino acid
33.319	Met
35.296	Lys
35.978	Ile
36.398	Leu
37.242	Phe

The sample chromatograms show high degree of overlap with the blank, indicating absence of significant quantities of amino acids.

Appendix 3. Mesocsom study data for biomass, tissue nutrient concentrations and fertilization

Biomass Total (g)

Monoculture

Treatment	F. pratensis	C. nigra
A	76.98	62.66
	73.35	52.28
	87.41	60.50
	73.14	47.60
5	**77.72**	**55.76**
SE	**3.35**	**3.52**
B	71.21	67.10
	77.50	76.72
	82.58	74.98
	72.93	68.31
15	**76.05**	**71.78**
SE	**2.55**	**2.39**
C	58.47	59.58
	71.24	69.44
	52.70	70.22
	64.75	64.91
25	**61.79**	**66.04**
SE	**4.00**	**2.45**
D	57.88	59.64
	66.44	63.28
	78.76	60.25
	62.31	52.45
35	**66.35**	**58.90**
SE	**4.49**	**2.29**
E	48.04	57.55
	47.57	55.16
	47.82	53.37
	54.92	55.62
45	**49.59**	**55.43**
SE	**1.78**	**0.86**

Mixture

Treatment	F. pratensis	C. nigra
A	40.11	32.94
	60.94	25.19
	55.06	31.03
	44.61	22.08
5	**50.18**	**27.81**
SE	**4.76**	**2.52**
B	47.38	27.98
	50.10	24.22
	44.26	25.57
	51.53	25.41
15	**48.32**	**25.80**
SE	**1.60**	**0.79**
C	62.68	16.57
	50.52	13.38
	51.94	13.72
	54.96	18.11
25	**55.03**	**15.44**
SE	**2.71**	**1.14**
D	48.69	9.02
	53.30	11.98
	52.66	4.29
	33.25	8.87
35	**46.98**	**8.54**
SE	**4.69**	**1.59**
E	48.40	9.31
	43.64	7.22
	43.59	6.15
	57.85	5.81
45	**48.37**	**7.12**
SE	**3.35**	**0.79**

Root:Shoot Ratio

Monoculture

Treatment	F. pratensis	C. nigra
A	0.118	0.256
	0.159	0.216
	0.113	0.207
	0.137	0.255
5	**0.132**	**0.234**
SE	**0.010**	**0.013**
B	0.078	0.288
	0.120	0.391
	0.105	0.192
	0.072	0.295
15	**0.094**	**0.291**
SE	**0.011**	**0.041**
C	0.068	0.241
	0.063	0.280
	0.052	0.279
	0.087	0.263
25	**0.067**	**0.266**
SE	**0.007**	**0.009**
D	0.056	0.249
	0.043	0.286
	0.037	0.365
	0.043	0.251
35	**0.045**	**0.288**
SE	**0.004**	**0.027**
E	0.038	0.189
	0.050	0.387
	0.025	0.214
	0.034	0.266
45	**0.037**	**0.264**
SE	**0.005**	**0.044**

Mixture

Treatment	F. pratensis	C. nigra
A	0.0523	0.2517
	0.0270	0.2177
	0.0119	0.0964
	0.0239	0.3247
5	**0.0288**	**0.2226**
SE	**0.0085**	**0.0476**
B	0.0205	0.2519
	0.0275	0.1816
	0.0220	0.1590
	0.0119	0.1787
15	**0.0205**	**0.1928**
SE	**0.0032**	**0.0203**
C	0.0112	0.3683
	0.0246	0.1795
	0.0178	0.1655
	0.0144	0.1116
25	**0.0170**	**0.2062**
SE	**0.0029**	**0.0560**
D	0.0255	0.3410
	0.0107	0.4212
	0.0176	0.2532
	0.0213	0.9537
35	**0.0188**	**0.4923**
SE	**0.0031**	**0.1576**
E	0.0219	0.4727
	0.0254	0.7604
	0.0213	0.0840
	0.0114	0.8857
45	**0.0200**	**0.5507**
SE	**0.0030**	**0.1780**

Shoot Potassium (%)

Monoculture

Treatment	F. pratensis	C. nigra
A	20.42	19.87
	13.48	13.02
	16.84	10.97
5	**16.91**	**14.62**
SE	**2.00**	**2.69**
B	31.91	14.36
	16.27	17.61
		10.82
	28.51	18.58
15	**25.56**	**15.34**
SE	**4.76**	**1.76**
C	22.19	
	27.73	10.24
	18.64	17.64
		23.19
25	**22.85**	**17.02**
SE	**2.65**	**3.76**
D	31.78	15.37
	36.63	12.09
		24.93
	16.51	18.80
35	**28.30**	**17.79**
SE	**6.07**	**2.74**
E	30.75	11.07
	15.31	23.96
	35.51	13.33
45	**27.19**	**16.12**
SE	**6.11**	**3.98**

Mixture

Treatment	F. pratensis	C. nigra
A	21.94	14.30
	27.48	15.31
	31.59	15.25
	14.19	10.57
5	**23.80**	**13.86**
SE	**3.76**	**1.12**
B	23.41	14.42
		13.36
	13.52	10.24
		17.71
15	**18.46**	**13.93**
SE	**4.95**	**1.54**
C	22.25	11.49
	17.42	9.08
	29.51	17.45
	42.12	17.74
25	**27.83**	**13.94**
SE	**5.37**	**2.17**
D	32.25	
	27.68	
	19.43	10.78
	31.80	18.60
35	**27.79**	**14.69**
SE	**2.97**	**3.91**
E	16.58	11.25
	36.90	
	25.69	
	26.37	11.65
45	**26.38**	**11.45**
SE	**4.16**	**0.20**

Shoot Phosphorus (%)

Monoculture

Treatment	F. pratensis	C. nigra
A	2.97	2.63
	2.64	2.66
	2.59	1.83
5	**2.73**	**2.37**
SE	**0.12**	**0.27**
B	3.73	2.66
	3.64	2.29
		2.05
	4.93	2.54
15	**4.10**	**2.39**
SE	**0.42**	**0.14**
C	6.51	
	5.80	2.15
	4.63	2.40
		2.34
25	**5.65**	**2.30**
SE	**0.55**	**0.08**
D	5.73	2.52
	6.26	1.85
		2.62
	5.39	2.18
35	**5.79**	**2.29**
SE	**0.25**	**0.17**
E		1.74
	5.22	2.46
	5.58	2.30
45	**5.40**	**2.17**
SE	**0.18**	**0.22**

Mixture

Treatment	F. pratensis	C. nigra
A	3.00	2.77
	3.04	2.81
	3.12	2.81
	2.64	2.73
5	**2.95**	**2.78**
SE	**0.11**	**0.02**
B	5.17	2.68
	4.51	2.58
	3.35	2.05
		2.45
15	**4.34**	**2.44**
SE	**0.53**	**0.14**
C	5.88	2.62
	4.80	
	5.52	2.65
	5.21	2.52
25	**5.35**	**2.60**
SE	**0.23**	**0.04**
D	5.89	
	5.62	
	4.85	2.81
	5.67	2.43
35	**5.51**	**2.62**
SE	**0.23**	**0.19**
E	4.87	1.82
	5.58	
	6.05	
	5.98	2.29
45	**5.62**	**2.06**
SE	**0.27**	**0.24**

Shoot Nitrogen (%)

Monoculture

Treatment	F. pratensis	C. nigra
A	1.091	1.991
	0.980	1.887
	1.095	1.997
	1.083	2.166
5	**1.062**	**2.010**
SE	**0.027**	**0.058**
B	1.204	1.748
	1.182	1.773
	1.076	1.777
	1.086	1.663
15	**1.137**	**1.740**
SE	**0.033**	**0.027**
C	1.102	1.771
	1.289	1.765
	1.363	2.175
	1.259	1.779
25	**1.253**	**1.873**
SE	**0.055**	**0.101**
D	1.253	1.346
	1.031	1.657
	1.299	1.649
	1.141	1.403
35	**1.181**	**1.514**
SE	**0.060**	**0.081**
E	1.253	1.529
	1.246	1.433
	1.245	1.389
	1.091	1.278
45	**1.209**	**1.407**
SE	**0.039**	**0.052**

Mixture

Treatment	F. pratensis	C. nigra
A	1.058	1.586
	1.073	1.670
	1.073	1.737
	1.085	1.643
5	**1.072**	**1.659**
SE	**0.006**	**0.031**
B	1.190	1.562
	1.207	1.843
	1.181	1.671
	1.139	1.607
15	**1.179**	**1.671**
SE	**0.014**	**0.062**
C	1.211	1.716
	1.124	
	1.249	1.613
	1.162	1.681
25	**1.187**	**1.670**
SE	**0.027**	**0.030**
D	1.447	1.608
	1.206	
	1.054	1.435
	1.143	1.579
35	**1.212**	**1.541**
SE	**0.084**	**0.054**
E	1.077	1.534
	1.011	1.486
	1.101	1.424
	1.048	1.426
45	**1.059**	**1.468**
SE	**0.019**	**0.027**

B. Data for mesocosm fertilization experiments

% N

Monoculture	% N			
Treatment	**S -**	**S +**	**C -**	**C +**
A	0.961	1.033	0.979	1.053
	1.034	1.092	0.945	1.047
	1.172	1.517	0.834	1.130
5	1.056	1.214	0.919	1.077
SE	0.062	0.153	0.044	0.027
E	0.890	1.205	1.300	1.148
	0.844	0.954	1.078	1.075
	0.730	1.506	0.976	1.326
45	0.821	1.222	1.118	1.183
SE	0.047	0.160	0.096	0.075

Mixture				
Treatment	**S -**	**S +**	**C -**	**C +**
A	0.916	1.045	1.146	1.177
	0.966	0.766	0.951	1.218
	0.686	1.364	1.116	1.272
5	0.856	1.058	1.071	1.222
SE	0.086	0.173	0.061	0.028
E	1.183	1.286	1.077	1.257
	1.321	1.328	0.914	1.193
	1.283	1.054	1.019	1.414
45	1.262	1.223	1.003	1.288
SE	0.041	0.085	0.048	0.066

Biomass

Monoculture	DM			
Treatment	**S -**	**S +**	**C -**	**C +**
A	27.290	40.780	27.400	33.170
	36.590	28.170	22.770	32.080
	34.470	31.400	27.300	34.200
5	32.783	33.450	25.823	33.150
SE	2.817	3.786	1.529	0.613
E	31.390	37.710	21.590	44.610
	27.080	37.880	18.900	32.890
	37.570	51.970	17.660	35.340
45	32.013	42.520	19.383	37.613
SE	3.048	4.731	1.161	3.573

Mixture				
Treatment	**S -**	**S +**	**C -**	**C +**
A	7.950	11.520	15.330	27.170
	11.590	7.690	19.680	19.740
	12.230	19.110	12.200	9.610
5	10.590	12.773	15.737	18.840
SE	1.334	3.360	2.171	5.095
E	30.950	37.590	5.230	7.300
	15.410	33.890	5.790	14.390
	13.460	24.920	8.110	11.060
45	19.940	32.133	6.377	10.917
SE	5.540	3.766	0.883	2.050

Key:

S = *Sanguisorba officinalis* + = fertilized

C = *Carex nigra* - = unfertilized

C. One way analyses of Variance for fertilization experiments.

% N

Fertilized

Monoculture

C. nigra

Effect	SS	DF	MS	F	P
WT	0.016960	1	0.016960	1.8032	0.250
Error	0.037623	4	0.009406		

S.officinalis

Effect	SS	DF	MS	F	P
WT	0.000090	1	0.000090	0.0012	0.974
Error	0.292062	4	0.073016		

Unfertilized

Monoculture

C. nigra

Effect	SS	DF	MS	F	P
WT	0.059183	1	0.059183	3.5694	0.132
Error	0.066322	4	0.016580		

S.officinalis

Effect	SS	DF	MS	F	P
WT	0.082274	1	0.082274	9.0244	0.040
Error	0.036468	4	0.009117		

Fertilized

Mixture

C. nigra

Effect	SS	DF	MS	F	P
WT	0.006468	1	0.006468	0.851	0.408
Error	0.030403	4	0.007601		

S.officinalis

Effect	SS	DF	MS	F	P
WT	0.040574	1	0.040574	0.7282	0.442
Error	0.222857	4	0.055714		

Unfertilized

Mixture

C. nigra

Effect	SS	DF	MS	F	P
WT	0.006888	1	0.006888	0.7724	0.429
Error	0.035673	4	0.008918		

S.officinalis

Effect	SS	DF	MS	F	P
WT	0.247620	1	0.247620	18.0577	0.0132
Error	0.054851	4	0.013713		

C. One way analyses of Variance for fertilization experiments.

Biomass

Fertilized

Monoculture

C. nigra

Effect	SS	DF	MS	F	P
WT	29.882	1	29.882	1.5192	0.285
Error	78.679	4	19.670		

S.officinalis

Effect	SS	DF	MS	F	P
WT	123.397	1	123.397	2.2459	0.208
Error	219.778	4	54.945		

Unfertilized

Monoculture

C. nigra

Effect	SS	DF	MS	F	P
WT	62.210	1	62.210	11.2791	0.0283
Error	22.062	4	5.516		

S.officinalis

Effect	SS	DF	MS	F	P
WT	0.889	1.000	0.889	0.034	0.862
Error	103.115	4.000	25.779		

Fertilized

Mixture

C. nigra

Effect	SS	DF	MS	F	P
WT	94.169	1	94.169	2.08619	0.222
Error	180.557	4	45.139		

S.officinalis

Effect	SS	DF	MS	F	P
WT	562.214	1	562.214	14.75070	0.0184
Error	152.458	4	38.114		

Unfertilized

Mixture

C. nigra

Effect	SS	DF	MS	F	P
WT	131.4144	1	131.4144	15.98388	0.0162
Error	32.8867	4	8.2217		

S.officinalis

Effect	SS	DF	MS	F	P
WT	131.134	1	131.134	2.69836	0.176
Error	194.391	4	48.598		

Appendix 4. Phospholipid Fatty Acid (PLFA) assay for microbial community composition.

Numbers 1-38 indicate individual fatty acids. Data is given as % composition. Letters A, B, C, D and E refer to mesocosm soils from water-table depth treatments of 5, 15, 25, 35 and 45 cm. The numbers next to the letters indicate replicate mesocosm soils respectively.

Sample\PLFA	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
A1	0.855156	0.943422	0.421417	1.659082	0.413845	2.981141	4.71591	0.778938	0.131968	0.603746	0.265533	0.17697	0.804929	1.135546	1.555956	9.90804	2.983583	11.95473	17.75847
A2	0.528514	0.651025	0.498862	2.067968	0.366838	2.049398	3.092958	0.587227	0.157301	0.486916	0.310555	0.180755	1.211866	0.755895	1.147832	7.255142	1.898599	11.31441	26.6439
A3	0.715607	0.939593	0.304308	1.746081	0.375089	3.314114	3.852354	0.50594	0.111005	0.56798	0.22963	0.132153	0.812	1.34413	1.572354	9.384195	3.844438	11.74232	18.53333
A4	0.799589	0.910229	0.359188	2.742318	0.301247	2.588192	3.384254	0.39935	0.147382	0.528584	0.155583	0.162704	0.871342	1.013231	1.556284	8.262352	2.613944	11.55428	19.62083
B1	0.609269	0.788855	0.489759	2.4142	0.510025	2.828667	3.400753	0.764166	0.149087	0.428582	0.427483	0.161012	1.013781	0.974865	1.531498	6.074857	3.292494	12.54985	28.00245
B2	0.648369	0.75478	0.541383	1.638029	0.297943	3.084781	3.39811	0.42998	0.145775	0.543119	0.213888	0.145411	0.833951	0.966805	1.57891	7.727564	4.015216	11.79629	20.82817
B3	0.508874	0.624713	0.276412	1.244806	0.30616	2.946147	3.168334	0.600188	0.121558	0.48013	0.321827	0.153061	1.294768	1.146681	1.610888	6.221335	4.593548	10.06906	30.66275
B4	0.363739	2.875741	0.320288	2.20973	0.277643	2.079914	2.55814	0.421477	0.146076	0.379947	0.204848	0.094815	1.025105	0.622789	1.689679	5.549658	3.081661	11.15434	30.7529
C1	0.729262	0.813635	0.362608	1.095307	0.841559	4.778806	3.442107	0.453669	0.103826	0.420824	0.354563	0.222776	0.58447	1.297239	1.916554	8.064218	7.270781	10.67455	11.89156
C2	0.574637	0.753513	0.167748	0.494365	0.681626	5.238008	3.162915	0.139968	0.065865	0.439961	0.18087	0.188869	0.25414	1.428072	1.937712	8.204452	8.433244	11.13659	11.12605
C3	0.67475	0.911811	0.36326	1.781361	0.858104	5.732556	3.471019	0.313434	0.151271	0.469324	0.227261	0.227148	0.694199	1.349449	2.242882	7.481672	7.128158	10.96916	12.39425
C4	0.593704	0.774604	0.22779	1.285156	0.612038	4.716202	3.150935	0.266725	0.10512	0.411135	0.199025	0.140108	0.586806	1.265411	2.226111	8.293134	6.776932	11.67676	15.87094
D1	0.713046	0.841938	0.250528	0.815024	0.589962	4.785559	3.140978	0.223295	0.101714	0.455252	0.210495	0.175904	0.556973	1.383211	1.896131	8.075579	6.829766	11.13383	15.95693
D2	0.801313	0.821404	0.244886	0.853352	0.904623	4.9365	3.361591	0.258168	0.060649	0.432437	0.269401	0.240864	0.669194	1.364923	2.079322	8.405685	6.998267	10.70545	12.93411
D3	0.698015	0.870671	0.175423	1.063354	0.787903	5.495172	3.334657	0.219944	0.103242	0.492144	0.317866	0.176615	0.630864	1.500498	1.946131	7.880958	8.675766	10.71999	13.32049
D4	0.621621	0.786806	0.387708	1.033799	0.593062	5.023954	3.056087	0.22917	0.09019	0.451028	0.184131	0.192908	0.573164	1.414929	1.793178	7.338108	7.590451	10.63181	12.7806
E1	0.735627	0.910477	0.346554	1.284897	0.660518	4.80228	3.02667	0.330375	0.081364	0.461615	0.182806	0.166534	0.463126	1.393692	1.813818	7.490892	6.550061	11.85421	12.03639
E2	0.74543	0.876295	0.189342	0.63924	0.824754	5.099651	3.23078	0.312524	0.124918	0.534616	0.178818	0.187972	0.429959	1.576378	1.751935	7.852169	7.449254	10.61345	12.36996
E3	0.675316	0.865889	0.291431	0.935159	0.763657	4.602259	2.933436	0.247046	0.133201	0.497	0.246087	0.148888	0.727624	1.218748	1.954769	7.952464	6.273145	11.21197	15.93113
E4	0.691127	0.879885	0.287982	1.238878	0.736534	5.051782	3.401558	0.188739	0.088173	0.467564	0.178862	0.175412	0.476946	1.436515	1.892158	7.664384	7.131884	11.38038	11.75229

	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38
A1	1.387189	1.026703	1.362126	2.688004	0.589453	0.620573	1.125539	4.561121	4.968308	9.158343	0.361873	0.137599	1.800573	2.085482	1.876092	1.589889	3.110616	0.920958	0.581176
A2	1.196746	0.582324	1.611021	1.9123	0.671727	0.566837	2.51412	3.647655	4.751916	7.860093	0.377343	0.220134	1.81102	2.705828	2.226825	2.161948	2.937129	0.752641	0.286429
A3	1.233518	1.19164	1.316923	2.373991	0.364916	0.439821	0.943015	5.725085	4.607355	8.699222	0.63482	0.179137	1.83008	2.636879	1.886387	1.388756	3.140742	0.967575	0.41352
A4	1.544205	0.736923	1.187622	2.03675	0.537771	0.449192	1.19585	3.265201	4.46862	9.63931	0.37119	0.277813	1.905754	3.449729	4.116073	2.251807	3.188576	1.148445	0.258295
B1	0.214199	0.661996	0.835723	1.040964	0.590225	0.411711	1.194474	3.766304	4.940613	6.634745	0.357773	0.222771	2.061337	2.979447	1.503108	2.049886	2.798204	0.770314	0.554552
B2	1.782776	0.971276	1.105637	2.077842	0.498787	0.415931	1.255509	3.964713	5.742561	9.924477	0.33058	0.319936	2.027848	2.175826	1.768262	1.28839	3.672244	0.763693	0.325241
B3	1.144208	1.153036	1.673871	1.540217	0.552758	0.416059	1.225884	3.883555	4.133638	6.732039	0.636201	0.186203	1.724192	1.874197	1.28651	1.345121	2.813349	0.849755	0.477962
B4	0.871517	0.641604	1.27468	1.179208	0.479626	0.27622	1.283882	2.875287	4.09662	6.976821	0.394718	0.135027	1.738595	3.238116	2.485399	2.60792	2.514707	0.768087	0.353475
C1	2.178129	1.203152	1.206903	1.923711	0.271683	0.515491	1.321112	4.91116	5.54092	11.48096	0.798417	0.606547	2.189819	1.212715	1.178993	0.877851	4.066363	2.684927	0.512839
C2	2.205122	1.349567	1.010428	2.240645	0.192835	0.428194	0.846026	6.110424	6.411171	11.61871	0.549266	0.568147	2.025469	1.585647	0.288894	0.4515	4.600569	2.584086	0.324689
C3	2.62794	1.379009	1.188328	2.032369	0.309831	0.432583	1.47742	4.259554	4.325412	9.560946	0.489225	0.519716	2.052471	2.368418	1.27553	1.121774	4.15282	2.513906	0.471679
C4	1.509108	1.200722	1.227905	2.148845	0.40934	0.56695	0.758236	3.937991	5.556079	10.92018	0.201062	0.343922	2.074813	1.722506	1.649	0.803079	4.091918	1.385539	0.31417
D1	1.9908	1.292999	1.192464	1.96799	0.32034	0.399852	1.440424	4.554434	6.985641	11.89348	0.424759	0.503677	2.147722	1.953082	0.727528	0.827053	0.331205	2.451577	0.458861
D2	1.949343	1.219557	1.136355	2.04225	0.287207	0.446812	1.045899	4.639993	6.052875	12.09073	0.207583	0.494439	1.99438	1.564952	0.947721	0.778571	4.314786	2.205189	0.239223
D3	1.72353	1.422204	0.757875	1.851636	0.261701	0.318673	1.28504	5.121615	5.610213	10.36262	0.902785	0.5143	1.833811	1.801905	0.921995	0.929553	4.072314	1.522326	0.376205
D4	2.963363	1.382846	1.027747	2.399667	0.260899	0.430646	1.084805	5.042449	5.85316	10.98963	0.752282	0.523865	1.869775	2.022404	0.841638	0.913334	4.622692	1.776886	0.469208
E1	1.919627	1.359555	1.154609	2.138405	0.310908	0.411246	1.071863	4.543812	7.494695	10.51441	0.517549	0.495126	1.9715	2.591762	1.278592	1.334436	4.124889	1.833618	0.341504
E2	2.669665	1.401313	1.206052	2.036615	0.29135	0.417417	0.884168	5.970057	6.538549	11.53275	0.732609	0.494395	2.02415	0.870304	0.707472	0.570564	4.433362	1.853004	0.37876
E3	1.153815	1.128564	0.955616	2.016718	0.232542	0.424714	1.143208	5.842078	7.05395	11.29739	0.50246	0.320647	1.855487	0.887624	0.544996	0.729692	3.959274	2.043228	0.298773
E4	2.033722	1.391492	1.152088	2.164922	0.259602	0.425968	1.087769	4.915566	6.358914	10.99964	0.40624	0.39924	1.950825	2.170916	1.311613	0.940573	4.458914	1.975265	0.475677

Table 4.1 Sample fatty acid elution order and retention times.

Fatty acid elution order	Fatty acid	Retention time / minutes
1	*i* 15:0	20.99
2	*ai* 15:0	21.28
3	15:0	22.28
4	2OH 14:0	22.98
5	16:1	23.84
6	*i* 16:0	24.61
7	*ai* 16:0	24.92
8	16:1ω9	25.05
9	16:1ω7 c	25.22
10	16:1ω7 t	25.37
11	16:1ω5	25.56
12	16:0	26.01
13	unknown (1)	27.28
14	Me 17:0 isomer (1)	27.39
15	Me 17:0 isomer (2)	27.57
16	17:1	27.66
17	Me 17:0 isomer (3)	27.83
18	unknown (2)	28.11
19	*i* 17:0	28.26
20	*ai* 17:0	28.59
21	unknown (3)	28.79
22	*cyc*-17:0	29.14
23	17:2	29.41
24	17:0	29.62
25	10 Me 17:0 (4)	29.75
26	Me 18:0 isomer	31.13
27	18:2ω6 c	32.18
28	18:1ω9 c	32.4
29	18:1ω9 t and C18:1ω7c	32.61
30	18:1ω7t	32.83
31	18:1	32.95
32	18:0	33.31
33	Me 19:0 isomer	33.55
34	19:2	34.72
35	*cyc*-19:0	36.51
36	unknown (4)	38.09
37	unknown (5)	38.37
38	20:0	40.35

Source:

Pawlett, M. (2004) *The interaction between earthworms, liming and soil microbial community diversity and function in upland grassland.* PhD Thesis. University of East London, UK.

Appendix 5. Field monitoring data from Cricklade North Meadow National Nature Reserve Soil water-table depth, nitrogen availability and above ground plant production data are shown.

Quadrat	Water-table depth / cm	soil nitrogen availabilty /log µmol bag^{-1}	Abovegrd Biomass / g m^{-2}	Plant tissue			
				Phosphorus / ‰	Nitrogen / %	Potassium / ‰	N:P ratio
3	46.393	1.813	589.614	2.921	2.176	8.201	7.448
5	62.623	1.962	267.972	2.682	2.149	12.842	8.014
7	66.484	0.822	349.071	1.791	1.632	6.032	9.112
9	68.402	1.232	322.739	2.356	1.824	11.582	7.743
13	54.956	1.549	309.220	1.816	1.914	7.304	10.541
15	46.530	1.437	369.414	3.056	2.001	12.761	6.549
17	57.468	1.574	327.151	1.833	1.817	5.248	9.913
19	64.608	1.070	340.654	2.383	1.764	14.397	7.402
23	27.968	2.541	529.812	2.680	2.540	11.842	9.479
25	30.093	2.484	436.274	2.677	2.076	14.140	7.756
27	55.845	1.602	418.559	2.001	1.913	8.402	9.558
29	60.375	0.781	258.839	2.061	1.827	10.758	8.864
33	25.318	2.021	470.910	2.753	2.621	9.633	9.520
35	41.576	1.455	468.478	2.686	2.260	9.648	8.413
37	35.729	1.882	528.810	2.124	2.076	9.959	9.775
39	40.327	2.855	412.813	2.802	2.030	13.367	7.246
43	45.538	2.068	282.375	1.368	1.513	7.219	11.058
45	39.438	0.951	463.809	2.679	1.961	9.338	7.321
47	57.330	0.838	477.867	2.400	2.276	7.947	9.483
49	48.363	0.770	327.844	2.211	1.802	11.377	8.151
53	61.962	1.211	454.102	1.510	1.737	8.541	11.505
55	48.355	1.887	394.938	2.255	2.055	10.990	9.111
57	47.786	1.563	416.571	1.783	2.006	6.180	11.248
59	49.652	1.203	255.502	2.200	1.961	11.377	8.912
63	65.600	1.628	403.293	1.522	1.660	10.057	10.907
65	52.498	1.781	437.912	2.049	1.963	10.137	9.579
67	44.740	2.012	397.142	1.926	2.112	9.599	10.966
69	64.164	1.480	368.200	1.930	1.857	13.293	9.623
73	60.205	1.434	422.189	1.761	1.906	9.419	10.823
75	48.972	2.096	519.359	1.950	1.748	11.130	8.963
77	45.623	1.892	439.594	1.901	1.949	8.628	10.252
79	59.807	1.887	530.588	1.889	1.832	15.386	9.701
83	65.518	1.232	415.171	1.614	2.034	11.111	12.605
85	43.268	1.401	285.367	1.985	1.856	8.871	9.350
87	47.028	1.520	359.558	1.473	1.618	7.516	10.981
89	60.183	1.017	373.969	2.350	2.031	13.647	8.645
93	52.881	1.479	329.522	1.470	1.435	9.252	9.763
95	54.073	1.890	337.912	1.938	1.826	13.280	9.420
97	46.997	1.812	435.839	1.675	1.990	9.371	11.883
99	59.330	1.154	408.263	1.943	1.900	16.049	9.776
103	58.109	1.490	312.013	1.731	1.972	7.329	11.392
105	57.776	1.063	491.839	2.577	1.656	11.582	6.426
107	54.497	1.448	558.255	1.461	1.806	10.188	12.360
109	62.110	1.366	392.460	1.860	1.755	14.089	9.436